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(54) LFA-3 protein and derivatives

1 FA-3-Protein und Derivate

LFA-3 protéine et dérivés

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Description

The present invention relates to a novel cell adhesion protein, a gene coding for the same, a process for preparing the same and a carrier onto which the same is immobilized.

Forming rosette with sheep erythrocytes has been recognized to be one of specific responses of human T-cells. At present, it is understood that the forming rosette of sheep erythrocytes and human T-cells is a binding response due to high affinity of CD2 antigen receptor on a sheep erythrocyte for CD2 antigen on human T-cells (another name: T11 antigen). Any clear answer has not been obtained for the question why human T-cells form rosette with sheep erythrocytes easily. However, there is a possibility that in the structure of a sheep receptor for CD2 antigen itself there exists a function different from the function of human LFA-3, being a receptor for CD2 antigen in human. At present, it is known that the partly determined N-terminal amino acid sequence of a receptor on a sheep erythrocyte for CD2 antigen (SEQ ID NO:3) has about 50 % homology with the amino acid sequence of human LFA-3, a receptor for CD2 antigen (refer to Unexamined Japanese Patent Publication No. 150228/1988). However, it is not known what kind of whole structure the receptor on sheep erythrocyte for CD2 antigen has. As to human LFA-3 molecule, it is known that the molecule is classified as a cell adhesion protein belonging to immunoglobulin superfamily (refer to A. F. Williams and A. N. Barclay, Annu. Rev. Immunol. 6, 381, (1988)) and that the molecule is constructed by, from N-terminus, immunoglobulin-like domain 1 (D1 region), immunoglobulin-like domain 2 (D2 region), transmembrane region (TM region) and cytoplasm region (C region) (refer to B. P. Wallner et al., J. Exp. Med. 166, 923, (1987)). Further, there is also known human LFA-3 molecule which has D1 region and D2 region and binds to a membrane through glycosyl phosphatidylinositol (refer to B. Seed, Nature 329, 840, (1987)). Therefore, in the present specification, a CD2 antigen receptor having a structure of, from N-terminus, D1 region - D2 region - TM region - C region or a CD2 antigen receptor having D1 region and D2 region and binding to a membrane through glycosyl phosphatidylinositol is referred to as "LFA-3". A sheep receptor for CD2 antigen having unknown structure is kept to be referred to as a receptor for CD2 antigen.

A sheep receptor for CD2 antigen has various uses, such as a use as a reagent for detecting T-cells and a use as a ligand for separating T-cells from a mixture of various kinds of cells, because the receptor has high affinity for CD2 antigen of human T-cells. Further, it is known that CD2 antigen participates in various immune responses as functions of T-cells. Therefore, a sheep receptor for CD2 antigen which has affinity for CD2 antigen can be used as an immunoregulatory agent and more, as a therapeutic agent which targets a tumor of T-cell family or a leukemia cell by utilizing affinity thereof for T-cells.

A sheep receptor for CD2 antigen can be obtained from sheep erythrocytes. As a process for preparing a sheep receptor for CD2 antigen and a derivative of the receptor, there are known a process comprising solubilizing the receptor from sheep erythrocytes by a surfactant and purifying by an affinity-chromatography using antibodies therefor (refer to Unexamined Japanese Patent Publication No. 150228/1988) and a process comprising solubilizing the receptor from sheep erythrocytes by trypsin (refer to T. Kitao et al., J. Immunol. 117, 310, (1976)). However, a amount of the sheep receptor for CD2 antigen is very small on sheep erythrocytes and it is hard work to prepare a large amount of the sheep receptor for CD2 antigen for the above-mentioned uses.

At present, a protein which naturally exists in very small amount can be prepared inexpensively and in large amount by genetic engineering techniques. For preparing the sheep receptor for CD2 antigen by genetic engineering techniques, a necessary gene coding for the sheep CD2 antigen receptor has to be isolated (cloned) first. However, at present, a whole amino acid sequence of the sheep receptor for CD2 antigen is not known. Further, it is not known at all whether the sheep receptor for CD2 antigen is LFA-3 which is a CD2 antigen receptor having the structure of, from N-terminus, D1 region - D2 region - TM region - C region or a CD2 antigen receptor having D1 region and D2 region and binding to a membrane through glycosyl phosphatidylinositol, or a receptor of other structures. Therefore, it has been impossible to detect a gene or mRNA coding for sheep LFA-3, to clone the sheep LFA-3 gene and to prepare sheep LFA-3 by genetic engineering techniques.

It can be considered that some processes can be used for cloning of sheep LFA-3 gene. For example, it may be possible, by using of DNA probe (mixed probe) which is deduced from the known N-terminal partial amino acid sequence of a sheep receptor for CD2 antigen (SEQ ID NO:3) consisting of 29 amino acid residues, to screen a cDNA of sheep LFA-3 from a cDNA library derived from cells in which the sheep LFA-3 gene is expressed. However, the cDNA screened by the mixed DNA probe can not be certified to be a true gene coding for sheep LFA-3 unless the cDNA is sequenced. A mixed DNA probe is not appropriate for detecting for the sheep LFA-3 gene. A DNA probe which is designed and prepared according to the Lathe et al.'s method (refer to Lathe et al., J. Molec. Biol. 183, 1, 1985) is not always useful to clone a gene.

A monoclonal antibody for a sheep receptor for CD2 antigen has been obtained. It may be possible that cloning of the desired gene can be attained by using the antibody labeled by a radioactive substance to screen the gene from a gene expression library. However, there is no report wherein the monoclonal antibody can be really used for cloning.

Therefore, a sure means by which the sheep LFA-3 gene can be cloned has not been accomplished at present.

A DNA probe which is useful for sure cloning of the sheep LFA-3 gene is a DNA probe which has the sequence of LFA-3 gene as it is. Such a DNA probe can selectively hybridize with the sheep LFA-3 gene or mRNA, therefore it is very useful to detect the LFA-3 gene or mRNA.

On the other hand, until now, there have not been known the existence of a LFA-3 like protein deficient in D2 region in sheep and also in humans. Such proteins have been first found in the present invention.

If these proteins have high affinity for CD2 antigen of human T-cells, it is considered that they are useful as a detecting reagent for human T-cells, as a ligand for separating T-cells of humans and the other animals, as an immuno-regulatory agent or as a therapeutic agent which targets tumors of T-cell family. For these uses, it is necessary to make a mass production of the protein possible. Although a process by genetic engineering techniques is appropriate for a mass production of such a protein, a gene coding for the protein has to be cloned and has to be analyzed in order to perform the process. Further, it is also necessary to search a protein which is more suitable for a process by genetic engineering techniques and to clone a gene coding for such a protein and to analyze the structure of the protein.

An object of the present invention is to provide a protein, which is appropriate for a process by genetic engineering techniques, having high affinity for CD2 antigen on human T-cells.

Another object of the present invention is to provide a gene coding for such a protein.

A further object of the present invention is to provide a process for preparing such a protein by genetic engineering techniques.

A still further object of the present invention is to provide a carrier onto which such a protein is immobilized.

These and the other objects of the present invention will become apparent from the description hereinafter.

In accordance with the present invention, there are provided a sheep LFA-3 like protein a human LFA-3 like protein, derivatives of these proteins, genes coding for the respective above-mentioned proteins, a process for preparing the proteins and a carrier onto which the respective proteins are immobilized.

Fig. 1 is a figure in which the amino acid sequence of human LFA-3 (SEQ ID NO:8) is compared with the amino acid sequence of the sheep LFA-3 like protein deficient in TM region (SEQ ID NO:36) (not included in the present invention). Number in the figure represents number of amino acid from the N-terminus of human LFA-3. A symbol "-" represents deficiency of amino acid. A colon is shown between homologous amino acids. TM region is underlined. As is clear from the figure, does sheep have the like protein deficient in TM region does not have the underlined sequence.

Fig. 2 is a figure in which the amino acid sequence of human LFA-3 (SEQ ID NO: 8) is compared with the amino acid sequence of the sheep LFA-3 like protein of claim 1, which is deficient in D2 region (SEQ ID NO:1). Number in the figure represents number of amino acid from the N-terminus of human LFA-3. A symbol "-" represents deficiency of amino acid. A colon is shown between homologous amino acids. D2 region is underlined. As is clear from the figure, the sheep LFA-3 like protein deficient in D2 region does not have the underlined sequence.

Fig. 3 is a graph showing rosette formation inhibition activity of the proteins of the present invention obtained in Example 4-r and Example 5-z respectively, that is, sheep D1HC protein and human D1HC protein.

A LFA-3 like protein of claim 1 is one protein of the present invention. The LFA-3 like protein of claim 1 is a single polypeptide having the molecular weight of about 15,000 as protein, which has structure of a LFA-3 protein lacking in D2 region and contains at most one disulfide bonding in the molecule. "D2 region" used herein means a region encoded by DNA which is segmented with introns from DNA encoding other regions on a genomic DNA, being the second immunoglobulin-like domain from N-terminus among two immunoglobulin-like domains of LFA-3 protein. The D2 regions in human and sheep have 6 cysteine residues.

According to the present invention, it has been revealed that the LFA-3 like protein having D1 region and no D2 region shows higher affinity for CD2 antigen in comparison with LFA-3. Such fact has demonstrated that capacity for adhere to CD2 antigen in LFA-3 exists in not D2 region but D1 region. Therefore, it is considered that LFA-3 protein wherein there is (are) introduced variation(s) such as partial deficiency and/or substitution in D2 region, has the similar affinity to that of the LFA-3 like protein of claim 1.

The LFA-3 like protein of the present invention has low molecular weight in of the present invention has low molecular weight in comparison with the molecular weight of LFA-3. The protein of the present invention has at most one disulfide bonding whereas LFA-3 has plural disulfide bondings in the molecule. Therefore, the protein of the present invention is very advantageous by reasons of the low antigenicity and no possibility of forming a wrong disulfide bonding in production by genetic engineering techniques.

Hereinafter there is explained a derivative of the above-mentioned protein of claim 1, which is a still further protein produced according to the present invention. The derivative of the present invention includes soluble derivatives of the LFA-3 like protein of claim 1 which are proteins wherein variation(s) such as deficiency and/or substitution is (are) introduced in TM region rich with hydrophobic amino acids and/or C region thereof. The soluble derivative of the LFA-3 like protein of claim 1 is very advantageous to production by genetic engineering techniques by reason that the derivative can be secreted from an animal cultured cell, a yeast or the like after production therein. Also, because D1 region of human and sheep LFA-3 has no cysteine residue, there can be produced the derivative having no cysteine residue or the derivative having at most two cysteine residues according to the present invention. Such derivatives are

very advantageous to production by genetic engineering techniques by reason of no possibility that a wrong disulfide bonding is formed.

From the derivative of the present invention wherein a few cysteine residues are introduced can be produced a carrier onto which the derivative is immobilized through the cysteine residue, a multimer and the derivative covalently bound with other substance. "D1 region" used herein means a region encoded by DNA which is segmented with an intron from the DNA encoding D2 region on a genomic DNA, being the immunoglobulin-like domain of N-terminus side among two immunoglobulin-like domains of LFA-3 protein.

The gene coding for the LFA-3 like protein and the above-mentioned protein are prepared as described below.

First, mRNA of the sheep LFA-3 like protein of claim 1 region is prepared. The mRNA of the sheep LFA-3 like protein of claim 1 can be extracted from sheep cells or a sheep organ wherin the gene of the sheep LFA-3 like protein of claim 1 has been expressed, such as leukocytes. Firstly, RNA is extracted from such a material by means of a conventional method such as guanidine thiocyanate method or hot phenol method. Then, from the extracted RNA, mRNA can be prepared as poly(A)+RNA by means of an oligo(dT)-cellulose column (refer to Rabomanyuaruidenshikogaku, edited by Masami Muramatsu, Maruzen, (1988)).

Second, cDNA of the sheep LFA-3 like protein of claim 1 is prepared from the obtained mRNA by means of a conventional method or a commercially available kit.

There are synthesized mixed primers each of which is deduced from amino terminal sequence and carboxyl terminal sequence of the known N-terminal 29 amino acid residues of the CD2 antigen receptor of sheep erythrocytes (SEQ ID NO: 3). With the synthesized mixed primers, the above obtained cDNA is amplified by PCR method. The amplification by PCR method may be cycled suitable times to obtain a sufficient amount of cDNA for the following cloning. Thereby is amplified cDNA coding for N-terminal portion of the sheep LFA-3 like protein deficient in D2 region. The amplified cDNA is detected by a conventional gel electrophoresis, e.g., polyacrylamide gel electrophoresis, and extracted.

Nucleotide sequence of the extracted DNA can be determined by using a suitable vector such as pBR322, pUC18 or pUC19, cloning the vector in a suitable host such as E. coli and utilizing a conventional method, e.g., Sanger method or the like.

There can be used DNA having the determined DNA sequence of the sheep LFA-3 like protein of claim 1 as a probe useful for detection of the gene of the sheep LFA-3 like protein of claim 1 and mRNA thereof. That is, a full-length cDNA of the sheep LFA-3 like protein of claim 1 can be easily detected by using this probe form the cDNA library. The cloned full-length cDNA of the sheep LFA-3 like protein of claim 1 is characterized by analyzing with restriction enzymes or sequencing thereof. On the basis of the obtained nucleotide sequence of the gene, amino acid sequence of the sheep LFA-3 like protein of claim 1 is determined.

The sheep LFA-3 like protein of claim 1 can be obtained by inserting thus obtained cDNA of the sheep LFA-3 like protein of claim 1 into a suitable expression vector, introducing the recombinant vector into a host cell suitable for the vector, for instance, a bacterium such as E. coli or Bacillus, a yeast, a fungus, a cultured cell of animal or plant or the like and culturing the transformed cell.

Also a soluble derivative of the sheep LFA-3 like protein of claim 1 can be prepared as a variant wherin variation(s) such as deficiency and/or substitution is (are) provided in the transmembrane region and/or the cytoplasm region whereas the amino acid sequence of D1 region is maintained, for instance, the derivative of the sheep LFA-3 like protein having an amino acid sequence wherin the sequence from the 1st amino acid to the 94th amino acid in SEQ ID NO: 1 is maintained and, in the sequence from the 95th amino acid to the 131st amino acid in SEQ ID NO: 1, a deficiency of at least one amino acid and/or a substitution of sequence exist(s), as defined in claim 5.

The human LFA-3 like protein of claim 2 is obtained described below.

First, mRNA of the human LFA-3 like protein of claim 2 is prepared. The mRNA of the human LFA-3 like protein of claim 2 can be extracted from human cells or a human organ wherin the gene of the human LFA-3 like protein of claim 2 has been expressed, such as leukocytes or an established strain of human T-cells. Firstly, RNA is extracted from such a material by means of a conventional method such as guanidine thiocyanate method or hot phenol method. Then, from the extracted RNA, mRNA can be prepared as poly(A)+RNA by means of an oligo(dT)-cellulose column (refer to Rabomanyuaruidenshikogaku, edited by Masami Muramatsu, Maruzen, (1988)).

Second, cDNA of the human LFA-3 like protein of claim 2 is prepared from the obtained mRNA by means of a conventional method or a commercially available kit.

There are synthesized 5' primer and 3' primer coding for amino terminal sequence or carboxyl terminal sequence of the known amino acid sequence of human LFA-3 (SEQ ID NO: 8). With the synthesized primers, the above obtained cDNA is amplified by PCR method. The amplification by PCR method may be cycled suitable times to obtain a sufficient amount of cDNA for the following cloning. The amplified cDNA is detected by a conventional gel electrophoresis, e.g., polyacrylamide gel electrophoresis, and extracted from the gel.

Nucleotide sequence of the extracted DNA can be determined by using a suitable vector such as pBR322, pUC18 or M13mp19, cloning the recombinant vector in a suitable host such as E. coli JM109, and using a conventional method,

e.g. Sanger method or the like.

Also, the DNA of the human LFA-3 like protein of claim 2 is screened from the human cDNA library as a clone which is selected with a probe having the nucleotide sequence of D1 region and is not selected with a probe having the nucleotide sequence of D2 region; and cloned.

5 The cloned cDNA of the human LFA-3 like protein of claim 2 is characterized by analyzing with restriction enzymes or sequencing thereof.

The human LFA-3 like protein of claim 2 can be obtained by using a suitable expression vector and a host cell suitable for the vector, for instance, a bacterium such as *E. coli* or *Bacillus*, a yeast, a fungus, a cultured cell of animal or plant or the like and culturing the transformed cell.

10 A soluble derivative of the human LFA-3 like protein of claim 2 is prepared as a variant wherein variation(s) such as deficiency and/or substitution is (are) provided in the transmembrane region and/or the cytoplasm region whereas the amino acid sequence of D1 region is maintained, for instance, the derivative of the human LFA-3 like protein of claim 2 having an amino acid sequence wherein the sequence from the 1st amino acid to the 94th amino acid in SEQ ID NO: 13 is maintained and, in the sequence from the 95th amino acid to the 134th amino acid in SEQ ID NO: 13, a

15 deficiency of at least one amino acid and/or a substitution exists as defined in claim 6, or the derivative of the human LFA-3 like protein of claim 2 having an amino acid sequence wherein the sequence from the 1st amino acid to the 93rd amino acid in SEQ ID NO: 13 is maintained and, in the sequence from the 94th amino acid to the 134th amino acid in SEQ ID NO: 13, a deficiency of at least one amino acid and/or a substitution exist(s), as defined in claim 7.

Genes coding for the LFA-3 like protein deficient in D2 region of animals other than human and sheep are selected
20 from cDNA clones which hybridize to the gene of the sheep LFA-3 like protein of claim 1 or the gene of the human LFA-3 like protein of claim 2 as cDNA having no DNA sequence coding for the sequence of D2 region.

By using thus obtained cDNA of the LFA-3 like protein deficient in D2 region of animals other than human and sheep, the protein corresponding to the selected cDNA and a derivative thereof can be obtained in the same manner as described above.

25 Further, the present invention has first revealed that a carrier onto which the LFA-3 like protein of claim 1 or 2 or a derivative thereof is immobilized can adsorb cells having CD2 antigen. It is found that the carrier onto which the LFA-3 like protein of claim 1 or 2 the derivative thereof can selectively adsorb cells having CD2 antigen such as human T-cells and other animals T-cells. Also, adsorbed cells can be easily separated from the carrier with trypsin. Therefore, there can be utilized the carrier onto which the LFA-3 like protein of claim 1 or 2 or the derivative thereof for selective
30 adsorption or separation of the T-cells.

As a carrier to be used for immobilization in the present invention, there can be used any of carriers onto which protein can be immobilized. Examples of the carrier to be used in the present invention are, for instance, plastic beads, plastic plates, plastic Schales and the like. The LFA-3 like protein of claim 1 or 2 or the derivative thereof can be immobilized by hydrophobic bonding, covalent bonding, ionic bonding or the like onto the carrier.

35 According to the present invention there can be obtained the LFA-3 like protein of claim 1 or 2 which are proteins having high affinity for CD2 antigen of human T-cells and are the LFA-3 like proteins suitable for production by genetic engineering techniques.

According to the present invention DNA coding for the above-mentioned proteins can be obtained. Therefore, the present invention enables to produce the LFA-3 like protein of claim 1 or 2 by genetic engineering techniques. Also,
40 the above-mentioned molecules, variants and the combined protein can be chemically or enzymatically modified to give derivatives. Thus obtained derivatives include the derivatives having different affinity for CD2 antigen being a natural ligand of LFA-3 from that of LFA-3.

45 There can be used as diagnostic agents T-cell detection the LFA-3 like protein of claim 1 or 2 and derivatives thereof which are produced by genetic engineering techniques and have affinity to human T-cells. As the diagnostic agents, there can be utilized the above-mentioned proteins and derivatives thereof which are labeled with an enzyme, a fluorescent agent or an isotope. Also, the carrier of the present invention onto which at least one of them is chemically or physically immobilized can be subjected to separation (isolation or removal) of T-cells. There can be used the LFA-3 like protein of claim 1 or 2 and derivatives thereof, and the proteins combined with other protein as therapeutic agents which target a tumor of T-cell family or a leucemia cell by utilizing affinity thereof for T-cells. For instance, the above-mentioned proteins, derivatives and the combined proteins which are conjugated with a toxin such as ricin, can be
50 used as therapeutic agents.

55 It is known that CD2 antigen being a natural ligand of LFA-3 participates in various immune response as function of T-cell. Therefore, there can be utilized the LFA-3 like protein of claim 1 or 2 a variant thereof, or a derivative thereof which has affinity to CD2 antigen as an agent for inhibiting or activating immune response(s), i.e. an immunoregulative agent.

The present invention is more specifically described and explained by means of the following Examples in which all percents are by weight unless otherwise noted. It is to be understood that the present invention is not limited to the Examples, and various changes and modifications may be made in the invention without departing from the spirit and

scope thereof.

Reference Example 1

5 a. Synthesis of primers used in polymerase chain reaction (PCR) for preparing cDNA of sheep LFA-3

As a partial amino acid sequence of a sheep receptor for CD2 antigen, has been disclosed the N-terminal amino acid sequence consisting of 29 amino acid residues shown in SEQ ID NO: 3 (refer to Japanese Unexamined Patent Publication No. 150228/1988). In order to use in PCR for preparing cDNA coding for sheep LFA-3, following two kinds 10 of mixed primers were synthesized by means of a DNA synthesizer (made by Applied Biosystems, Model 381A). One mixed primer is shown in SEQ ID NO: 4, consisting of a restriction enzyme BamHI recognition sequence and a following nucleotide sequence deduced from the sequence of the 1st-7th amino acids in SEQ ID NO:3. The other is shown in SEQ ID NO: 5, consisting of a sequence containing a restriction enzyme PstI recognition sequence and a following nucleotide sequence deduced from the sequence of the 27th-22nd amino acids in SEQ ID NO:3.

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b. Preparation of double strand cDNA from sheep cells

From sheep was collected 100 ml of blood with heparin. The collected blood was centrifuged at 350G, 10 minutes to give a buffy coat fraction. After lysis of erythrocytes in the fraction by using erythrocyte lysing buffer, lysate was 20 washed twice with PBS (phosphate buffered saline) to give sheep leukocytes. Then, from the obtained sheep leukocytes was extracted RNA by guanidine thiocyanate method. Further poly(A)⁺RNA was purified by means of an oligo(dT)-cellulose column (refer to Raboman'yuaruidenshikogaku, edited by Masami Muramatsu, Maruzen, 1988). Double strand cDNA was synthesized from the poly(A)⁺RNA by means of a commercially available kit (You-Prime cDNA Synthesis Kit #27-9260-01, made by Pharmacia). Similarly, another double strand cDNA was synthesized from a commercially 25 available mRNA of sheep liver (made by Clontech).

c. Amplification by PCR method and cloning of cDNA coding for sheep LFA-3

By PCR method were amplified cDNA fragments coding for sheep LFA-3 in vitro. That is, using 100 µl of a reaction mixture containing 10 mM Tris-HCl (pH 8.3), 100 mM potassium chloride, 1.5 mM magnesium chloride, 0.01 % gelatin, 30 10 µM of each of 2 kinds of the mixed primers synthesized in the above-mentioned a, 10 ng of cDNA of the sheep leukocytes prepared in the above-mentioned b or cDNA of sheep liver, 0.2 mM of 4 kinds of deoxyribonucleic triphosphates (4 dNTP) and 2.5 units of Taq DNA polymerase, 35 cycles of PCR were carried out under the reaction condition per cycle of 94°C, 1 minute; 37°C, 2 minutes; and 72°C, 2 minutes. After completing the reactions, size of PCR products 35 was measured by a polyacrylamide gel electrophoresis, and then it was found that DNA fragments of about 100 base pairs were amplified in both cases of using cDNA of sheep leukocyte and using cDNA of sheep liver. The DNA fragments of about 100 base pairs were extracted from the gel and treated with restriction enzymes BamHI and PstI. Then the treated DNA was inserted into BamHI-PstI site of M13mp19 phage vector (made by TAKARA SHUZO Co.) and cloned by using E. coli JM109 as host.

40

d. Sequencing of DNA amplified by PCR

There was determined sequence of the DNA of about 100 base pairs prepared from a positive clone obtained in the above-mentioned c by a conventional method using dideoxynucleotide triphosphates. As a result, both sequences 45 between 2 kinds of the mixed primers used in PCR of PCR products from the leukocytes cDNA and the liver cDNA were determined to be the nucleotide sequence of the 19th-66th DNA sequence shown in SEQ ID NO: 30 (2 and 35). The sequence corresponds to the sequence from the 7th amino acid of Gly to the 22nd amino acid of Pro in the known partial amino acid sequence of a sheep receptor for CD2 antigen shown in SEQ ID NO: 3. It is shown that cDNA of sheep LFA-3 contains the above-mentioned nucleotide sequence. The DNA sequence codes the 7th-22nd amino acids 50 in SEQ ID NO: 31 (1 and 36).

e. Isolation of cDNA of sheep LFA-3 and analogues thereof

In order to clone a full-length cDNA of sheep LFA-3, sheep cDNA library was screened by using N-terminal cDNA 55 sequence of sheep LFA-3 determined in the above-mentioned d as a probe. That is, the double strand cDNA from sheep leukocytes prepared in the above-mentioned b was treated with DNA polymerase to give DNA fragments with blunt ends to which EcoRI linker (made by Pharmacia) was connected. Further, the linked DNA fragment was cut with EcoRI, and thereto were connected right and left arms of λ gt11 (made by Stratagene) treated with alkaline phosphatase.

Packaging in vitro was carried out to prepare cDNA library. There were synthesized probes having cDNA sequence adjacent N-terminus of sheep LFA-3, i.e., the probe having the nucleotide sequence of SEQ ID NO: 6 and that of SEQ ID NO: 7. By using these probes were screened about 2×10^5 recombinant phages. As a result, 3 positive clone (SL-6, SL-40 and SL-43) containing 1.0 kb (kilonucleotide)-1.2 kb of cDNA insert were obtained.

5

Example 2

m. Sequencing of cDNA of the sheep LFA-3 like protein of claim 1 (hereinafter referred to as "sheep Δ D2 protein")

10 Among the positive clones obtained in Reference Example 1, cDNA sequence contained in SL-40 was determined by dideoxy method using M13 phage. As a result, it is revealed that sheep Δ D2 protein is encoded by the nucleotide sequence of SEQ ID NO: 2 and has the amino acid sequence of SEQ ID NO: 1. It is also revealed that sheep Δ D2 protein has a signal peptide having 28 amino acid residues which starts from methionine. On the basis of thus obtained information of cDNA of sheep Δ D2 protein, natural type sheep Δ D2 protein and derivatives thereof can be produced
15 by genetic engineering techniques. The amino acid sequence of SEQ ID NO: 8 is that of human LFA-3 which has been reported by Wallner et al (B. P. Wallner et al., Journal of Experimental Medicine, vol. 166, p 923, (1987)), and SEQ ID NO: 16 shows the DNA sequence thereof. Also, Fig. 2 shows correspondence of the amino acid sequence of sheep Δ D2 protein shown in SEQ ID NO: 1 to that of human LFA-3 shown in SEQ ID NO: 8. Fig. 2 reveals that sheep Δ D2 protein lacks D2 region (underlined portion) existing in human LFA-3.

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n. Preparation of an expression vector of sheep Δ D2 protein in E. coli

In order to make cDNA coding for sheep Δ D2 protein express in E. coli, an expression vector was constructed.
25 The DNA insert contained in the cDNA clone SL-40 obtained in Reference Example 1 was taken out by cleaving with restriction enzyme EcoRI. The DNA fragment was subcloned into EcoRI site of plasmid pUC18. Successively such plasmid was subjected to PCR. Used 5' primer is shown in SEQ ID NO: 9. This primer is comprised of BamHI recognition sequence, Ncol recognition sequence and the DNA sequence designed according to the sequence of the 1st-7th amino acids of SEQ ID NO: 2. Used 3' primer is shown in SEQ ID NO: 10. The primer is comprised of Sall recognition sequence, PstI recognition sequence, sequence of termination codon and the DNA sequence designed according to the sequence
30 of the 131st-126th amino acids of SEQ ID NO: 2. PCR was carried out by using the primers of SEQ ID NO: 9 and SEQ ID NO: 10 to amplify DNA fragments. Thus amplified DNA fragments were cleaved with restriction enzymes BamHI and Sall, and thus obtained fragments were inserted into BamHI-Sall site of M13mp19 phage vector. Nucleotide sequence of the inserted DNA was confirmed by dideoxy method. Then, DNA coding for sheep Δ D2 protein was taken out from the M13mp19 phage vector by cleaving with restriction enzymes Ncol and PstI. Thus obtained DNA was
35 connected to Ncol-PstI site of vector pKK233-2 (made by Pharmacia) for expression to give an expression vector. Thus obtained expression vector is referred to as "pKSL Δ D2". SEQ ID NO: 20 shows nucleotide sequence from initiation codon to termination codon coding for sheep Δ D2 protein contained in pKSL Δ D2.

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o. Production of sheep Δ D2 protein by using E. coli as host

E. coli JM 109 (made by TAKARA SHUZO Co.) was precultured which has the expression vector pKSL Δ D2 of sheep Δ D2 protein. Then, the precultured E. coli was inoculated in 100 mL of LB medium containing 10 mg of ampicilline, and shaking culture was carried out at 37°C in 500 mL of Sakaguchi flask until A_{600} of the medium containing E. coli became 0.3. Successively thereto was added IPTG so as to give a final concentration of 1 mM and further culture was continued for 6 hours. The cultured cells were collected by centrifugation, and suspended in 10 mL of 50 mM Tris-HCl buffer (pH 8.0) containing 50 mM EDTA, 5 % Triton X-100 and 8 % sucrose. Thereto was added lysozyme so as to give a final concentration of 0.1 %. The cell suspension was sonicated, and centrifuged to give an insoluble precipitation fraction. The obtained precipitation was washed with 50 % glycerol and successively with ethanol to give inclusion bodies containing produced sheep Δ D2 protein. The inclusion bodies were dissolved with SDS and subjected to SDS-polyacrylamide gel electrophoresis to detect a band of molecular weight 15,000-16,000.

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p. Preparation of an expression vector of a soluble derivative of sheep Δ D2 protein (hereinafter referred to as "sheep D1HC protein") in E. coli

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In order to make cDNA of sheep D1HC protein express in E. coli, an expression vector was constructed.

Namely, the DNA insert contained in the cDNA clone SL-40 obtained in Reference Example 1 was taken out by cleaving with restriction enzyme EcoRI. The DNA insert was subcloned into EcoRI site of plasmid pUC18. Successively such plasmid was subjected to PCR. Used 5' primer is shown in SEQ ID NO: 9. This primer is comprised of BamHI

recognition sequence, Ncol recognition sequence and the DNA sequence designed according to the sequence of the 1st-7th amino acids of SEQ ID NO: 2. Used 3' primer is shown in SEQ ID NO: 11. The primer is comprised of PstI recognition sequence, sequence of termination codon, the 371st-358th nucleotide sequence and the 301st-277th nucleotide sequence of SEQ ID NO: 2. PCR was carried out by using the primers of SEQ ID NO: 9 and SEQ ID NO: 11 to amplify DNA fragments. Thus amplified DNA fragments were cleaved with restriction enzymes BamHI and PstI, and thus obtained fragments were inserted into BamHI-PstI site of M13mp19 phage vectors. Nucleotide sequence of the inserted DNA was confirmed by dideoxy method. Then, DNA of sheep D1HC protein was taken out from the M13mp19 phage vector by cleaving with restriction enzymes Ncol and PstI. Thus obtained DNA was connected to Ncol-PstI site of vector pKK233-2 (made by Pharmacia) for expression to give an expression vector. Thus obtained expression vector is referred to as "pKSLD1HC". SEQ ID NO: 21 shows nucleotide sequence from initiation codon to termination codon coding for sheep D1HC protein contained in pKSLD1HC.

q. Production of sheep D1HC protein by using E. coli as host

E. coli JM 109 (made by TAKARA SHUZO Co.) was precultured which has the expression vector pKSLD1HC of sheep D1HC protein. Then, the precultured E. coli was inoculated in 100 mL of LB medium containing 10 mg of ampicilline, and shaking culture was carried out at 37°C in 500 mL of Sakaguchi flask until A_{600} of the medium containing E. coli became 0.3. Successively thereto was added IPTG so as to give a final concentration of 1 mM and further culture was continued for 6 hours. The cultured cells were collected by centrifugation, and suspended in 10 mL of 50 mM Tris-HCl buffer (pH 8.0) containing 50 mM EDTA, 5 % Triton X-100, 2 mM dithiothreitol and 8 % sucrose. Thereto was added lysozyme so as to give a final concentration of 0.1 %. The cell suspension was sonicated, and centrifuged to give insoluble inclusion bodies. The inclusion bodies were dissolved with SDS and subjected to SDS-polyacrylamide gel electrophoresis to detect a band of molecular weight about 12,000.

r. Solubilization and renaturation of inclusion bodies containing sheep D1HC protein

All the inclusion bodies obtained in the above-mentioned q were dissolved in 10 mL of 50 mM Tris-HCl buffer (pH 7.4) containing 6M guanidine chloride and 2 mM EDTA, and centrifuged to give a supernatant. The supernatant was dialyzed against PBS to give a solution containing sheep D1HC protein. Thus prepared sheep D1HC protein was mixed with 1×10^5 Jurkat cells which were washed with the PBS containing 5 % bovine serum albumin (BSA) and 1 % glucose, and contained in 50 μ L of the same buffer. Thereafter there was observed effect on rosette formation by adding 1×10^7 sheep erythrocytes. Sheep D1HC protein dose-dependently inhibited the rosette formation of Jurkat cells with sheep erythrocytes. The results are shown in Fig. 3.

Rosette formation inhibition rate was calculated by the following formula;

$$\text{Inhibition rate (\%)} = 1 - \frac{\text{Rosette positive cells (\%)} \text{ in sample}}{\text{Rosette positive cells (\%)} \text{ in control}} \times 100$$

"Rosette positive cells" are cells to which not less than 5 sheep erythrocytes per cell adhere to form rosette.

Example 3

s. Synthesis of primers used in PCR for preparing cDNA of the human LFA-3 like protein of claim 2 (hereinafter referred to as "human Δ D2 protein")

There was cloned cDNA of human LFA-3, and the nucleotide sequence thereof has been known (B. P. Wallner et al., Journal of Experimental Medicine, vol. 166, p 923, (1987)). The nucleotide sequence is shown in SEQ ID NO: 16. In order to use in PCR for preparing cDNA coding for human Δ D2 protein, following 2 kinds of primers were synthesized by means of a DNA synthesizer (made by Applied Biosystems, Model 381A). One was a primer, shown in SEQ ID NO: 14, consisting of a sequence containing the recognition sequences of restriction enzymes PstI and Ncol and the 1st-24th nucleotide sequence of SEQ ID NO: 16. The other was a primer, shown in SEQ ID NO: 15, consisting of a sequence containing the recognition sequences of restriction enzymes PstI and EcoRI and the 753rd-730th nucleotide sequence of SEQ ID NO: 16.

t. Preparation of cDNA of human Δ D2 protein

Human T-cell line MOLT-4 (ATCC CRL-1582) was cultured in RPMI1640 medium containing 10 % fetal calf serum (FCS) to give 5×10^8 cells. washed twice PBS. Sucessively RNA was extracted from the cells by quanidine thiocyanate

method. Further poly(A)⁺RNA was purified by means of an oligo(dT)-cellulose column (refer to Rabomanyuaruiden-shikogaku, edited by Masami Muramatsu, Maruzen, (1988)). Double strand cDNA was synthesized from the poly(A)⁺RNA by means of a commercially available kit (cDNA synthesis kit # 27-9260-01, made by Pharmacia).

5 u. Amplification by PCR method and cloning of cDNA coding for human ΔD2 protein, and sequencing thereof

By PCR method were amplified cDNA fragments coding for human ΔD2 protein in vitro. That is, using 100 μl of a reaction mixture containing 10 mM Tris-HCl (pH 8.3), 100 mM potassium chloride, 1.5 mM magnesium chloride, 0.01 % gelatin, 10 μM of each of 2 kinds of primers synthesized in the above-mentioned s, 10 ng of human double strand 10 cDNA prepared in the above-mentioned t, 0.2 mM of 4 kinds of deoxyribonucleic triphosphates (4 dNTP) and 2.5 units of Taq DNA polymerase, 35 cycles of PCR were carried out under the reaction condition per cycle of 94°C, 1 minute; 37°C, 2 minutes; and 72°C 2 minutes. After completing the reactions, size of PCR products was measured by a poly-acrylamides gel electrophoresis, and then it was found that DNA fragments of about 500 base pairs were amplified. The DNA fragments of about 500 base pairs were extracted from the gel and treated with restriction enzyme PstI. Then 15 the treated DNA was inserted into PstI site of pUC19 vector (made by TAKARA SHUZO Co.) and cloned by using E. coli JM109 as host. The prepared plasmid is referred to as "pHLΔD2".

Then, in order to sequence cDNA amplified by PCR, the DNA fragment of about 500 base pairs cleaved with PstI was inserted into PstI site of M13mp19 phage vector (made by TAKARA SHUZO Co.) and sequenced by a conventional 20 method using dideoxynucleotide triphosphates. As a result, cDNA sequence of human ΔD2 protein shown in SEQ ID NO: 12 was found among about 500 base pairs of DNA fragments. Such cDNA sequence codes for the protein having the amino acid sequence shown in SEQ ID NO: 13. Comparing human ΔD2 protein shown in SEQ ID NO: 13 with human LFA-3 shown in SEQ ID NO: 8, it is found that human ΔD2 protein lacks the amino acids from the 94th amino acid of Glu to the 181st amino acid of Ser in SEQ ID No: 8, i.e., D2 region.

25 v. Preparation of an expression vector of human ΔD2 protein in E. coli

In order to make cDNA of human ΔD2 protein express in E. coli, an expression vector was constructed. The DNA 30 of plasmid pHLΔD2 having cDNA of human ΔD2 protein obtained in the above-mentioned u was used as a template to carry out PCR. Used 5' primer is shown in SEQ ID NO: 17. This primer is comprised of KpnI recognition sequence, GG, sequence of initiation codon and the 1st-24th nucleotide sequence of SEQ ID NO: 12. Used 3' primer is shown in 35 SEQ ID NO: 18. The primer is comprised of HindIII recognition sequence, NheI recognition sequence, sequence of termination codon and the 402nd-379th nucleotide sequence of SEQ ID NO: 12. PCR was carried out by using the primers of SEQ ID NO: 17 and SEQ ID NO: 18 to amplify DNA fragments. Thus amplified DNA fragments were cleaved with restriction enzymes KpnI and HindIII. The cleaved fragments were connected to KpnI-HindIII site of vector 40 pKK233Kpn to give an expression vector. Thus obtained expression vector is referred to as "pKHLΔD2". The vector pKK233Kpn is a plasmid wherein the sequence from SD sequence to initiation codon AGGAAACAGACCATG of pKK233-2 (made by Pharmacia) has been varied to the sequence AGGAGGTACCGGGATG containing the recognition sequence of restriction enzyme KpnI by site directed mutagenesis method. SEQ ID NO: 22 shows the nucleotide sequence from initiation codon to termination codon coding for human ΔD2 protein contained in pKHLΔD2.

45 w. Production of human ΔD2 protein by using E. coli as host

E. coli JM 109 (made by TAKARA SHUZO Co.) was precultured which has the expression vector pKHLΔD2 of 50 human ΔD2 protein. Then, the precultured E. coli was inoculated in 100 ml of LB medium containing 10 mg of ampicillin, and shaking culture was carried out at 37°C in 500 ml of Sakaguchi flask until A₆₀₀ of the medium containing E. coli became 0.3. Successively thereto was added IPTG so as to give a final concentration of 1 mM and further culture was continued for 6 hours. The cultured cells were collected by centrifugation, and suspended in 10 ml of 50 mM Tris-HCl buffer (pH 8.0) containing 50 mM EDTA, 5 % Triton X-100 and 8 % sucrose. Thereto was added lysozyme 55 so as to give a final concentration of 0.1 %. The cell suspension was sonicated, and centrifuged to give an insoluble precipitation fraction. The obtained precipitation was washed with 50 % glycerol and successively with ethanol to give inclusion bodies containing produced human ΔD2 protein. The inclusion bodies were dissolved with SDS and subjected to SDS-polyacrylamide gel electrophoresis to detect a band of molecular weight 15,000-16,000.

x. Preparation of an expression vector of a soluble derivative of human ΔD2 protein (hereinafter referred to as "human D1HC protein") in E. coli

In order to make cDNA of human D1HC protein express in E. coli, an expression vector was constructed. That is, the DNA of plasmid pHLΔD2 having cDNA of human ΔD2 protein obtained in the above-mentioned u was

used as a template to carry out PCR. Used 5' primer is shown in SEQ ID NO: 17. This primer is comprised of KpnI recognition sequence, GG, sequence of initiation codon and the 1st-24th nucleotide sequence of SEQ ID NO: 12. Used 5' primer is shown in SEQ ID NO: 19. The primer is comprised of HindIII recognition sequence, sequence of termination codon and the 402nd-373rd nucleotide sequence and the 297th-268th nucleotide sequence of SEQ ID NO: 12. PCR was carried out by using the primers of SEQ ID NO: 17 and SEQ ID NO: 19 to amplify DNA fragments. Thus amplified DNA fragments were cleaved with restriction enzymes KpnI and HindIII. The cleaved fragments were connected to KpnI-HindIII site of vector pKK233Kpn to give an expression vector. Thus obtained expression vector is referred to as "pKHLD1HC". SEQ ID NO: 23 shows the nucleotide sequence from initiation codon to termination codon coding for human D1HC protein contained in pKHLD1HC.

10 y. Production of human D1HC protein by using E. coli as host

E. coli JM 109 (made by TAKARA SHUZO Co.) was precultured which has the expression vector pKHLD1HC of 15 human D1HC protein. Then, the precultured E. coli was inoculated in 100 ml of LB medium containing 10 mg of ampicilline, and shaking culture was carried out at 37°C in 500 ml of Sakaguchi flask until A_{600} of the medium containing E. coli became 0.3. Successively thereto was added IPTG so as to give a final concentration of 1 mM and further culture was continued for 6 hours. The cultured cells were collected by centrifugation, and suspended in 10 ml of 50 mM Tris-HCl buffer (pH 8.0) containing 50 mM EDTA, 5% Triton X-100, 2 mM dithiothreitol and 8% sucrose. Thereto 20 was added lysozyme so as to give a final concentration of 0.1%. The cell suspension was sonicated, and centrifuged to give insoluble inclusion bodies. The inclusion bodies were dissolved with SDS and subjected to SDS-polyacrylamide gel electrophoresis to detect a band of molecular weight about 13,000.

z. Solubilization and renaturation of inclusion bodies containing human D1HC protein

All the inclusion bodies obtained in the above-mentioned y were dissolved in 10 ml of 50 mM Tris-HCl buffer (pH 25 7.4) containing 6M guanidine hydrochloride and 2 mM EDTA, and centrifuged to give a supernatant. The supernatant was dialyzed against PBS to give a solution containing human D1HC protein. Thus prepared human D1HC protein in an amount ranging from 2.5-0 µg was mixed with 1×10^5 Jurkat cells which were washed with the PBS containing 5 % BSA and 1% glucose, and contained in 50 µl of the same buffer. Thereafter there was observed effect on rosette formation by adding 1×10^7 sheep erythrocytes. Human D1HC protein dose-dependently inhibited the rosette formation 30 of Jurkat cells with sheep erythrocytes. The results are shown in Fig. 3.

Example 4

35 α. Immobilization of sheep or human D1HC protein onto carrier

In 0.1 M glycine buffer (pH 8.2) containing 15 mM NaCl was dissolved sheep D1HC protein prepared in the above-mentioned r or human D1HC protein prepared in the above-mentioned z so as to give 100 µg/ml of final concentration thereof. Thus obtained solution was added in an amount of 100 µl per well to 96-wells microtiter plate made by Costar 40 (catalog number 3590), and incubated at 37°C for 1 hour to coat bottom of wells in plate with the protein. Then, the plate was treated with 1% solution of BSA. After washing the plate thereto were added 2×10^5 Jurkat cells per well which were washed with the PBS containing 5% BSA and 1% glucose and suspended in 100 µl of the same buffer. The plate was allowed to stand for 30 minutes at 4°C. The plate was washed 3 times with PBS, then cells adhered to 45 the bottom of wells were observed. As a result, it is found that the cells adhered over the bottom of wells coated with sheep D1HC protein or human D1HC protein. In contrast, the cells hardly adhered to the bottom of wells coated with no D1HC protein.

Example 5

50 β. Preparation of an expression vector of sheep D1HC protein containing cysteine residue (hereinafter referred to as "sheep D1HC cys protein") in E. coli

In order to make cDNA of sheep D1HC protein having cysteine residue at carboxyl terminal express in E. coli, an expression vector was constructed. The DNA of plasmid pKSLD1HC having cDNA of sheep D1HC protein obtained 55 in the above-mentioned p was used as a template to carry out PCR. Used 5' primer is the primer used in the above-mentioned p and shown in SEQ ID NO: 9. Used 3' primer is shown in SEQ ID NO: 24. The primer is comprised of HindIII recognition sequence, PstI recognition sequence, sequence of termination codon, sequence coding for cysteine residue and then the following 318th-295th nucleotide sequence of SEQ ID NO: 21. PCR was carried out by using the

primers of SEQ ID NO: 9 and SEQ ID NO: 24 to amplify DNA fragments. Thus amplified DNA fragments were cleaved with restriction enzymes BamHI and PstI. The cleaved fragment was inserted into BamHI-PstI site of M13mp19 phage vector to give a recombinant vector. Nucleotide sequence of DNA introduced in the recombinant vector was confirmed by dideoxy method. Then, cDNA coding for sheep D1HCcys protein was taken out from the M13mp19 phage vector by cleaving with restriction enzymes NcoI and PstI. Thus obtained DNA was connected to NcoI-PstI site of vector pKK233-2 (made by Pharmacia) to give an expression vector pKSLD1HCcys. SEQ ID NO: 25 shows the nucleotide sequence from initiation codon to termination codon coding for sheep D1HCcys protein contained in pKSLD1HCcys.

γ . Production of sheep D1HCcys protein by using E. coli as host

E. coli JM 109 (made by TAKARA SHUZO Co.) was precultured which has the expression vector pKSLD1HCcys of sheep D1HCcys protein. Then, the precultured E. coli was inoculated in 100 mL of LB medium containing 10 mg of ampicilline, and shaking culture was carried out at 37°C in 500 mL of Sakaguchi flask until A_{600} of the medium containing E. coli became 0.3. Successively thereto was added IPTG so as to give a final concentration of 1 mM and further culture was continued for 6 hours. The cultured cells were collected by centrifugation, and suspended in 10 mL of 50 mM Tris-HCl buffer (pH 8.0) containing 50 mM EDTA, 5 % Triton X-100, 2 mM dithiothreitol and 8 % sucrose. Thereto was added lysozyme so as to give a final concentration of 0.1 %. The cell suspension was sonicated, and centrifuged to give insoluble inclusion bodies. The obtained inclusion bodies were dissolved with SDS and subjected to SDS-polyacrylamide gel electrophoresis to detect a band of molecular weight about 13,000.

δ . Solubilization and renaturation of inclusion bodies containing sheep D1HCcys protein

All the inclusion bodies obtained in the above-mentioned γ were dissolved in 10 mL of 50 mM Tris-HCl buffer (pH 7.4) containing 6M guanidine hydrochloride, 2 mM EDTA and 5 mM 2-mercaptoethanol, and centrifuged to give a supernatant. The supernatant was dialyzed against PBS to give a solution containing sheep D1HCcys protein. Thus prepared sheep D1HCcys protein in an amount ranging from 2.5-0 μ g was mixed with 1×10^5 Jurkat cells which were washed with the PBS containing 5 % BSA and 1 % glucose, and contained in 50 μ L of the same buffer. Thereafter there was observed effect on rosette formation by adding 1×10^7 sheep erythrocytes. Sheep D1HCcys protein dose-dependently inhibited the rosette formation of Jurkat cells with sheep erythrocytes.

Example 6

ε . Preparation of an expression vector of human D1HC protein containing cysteine residue (hereinafter referred to as "human D1HCcys protein") in E. coli

In order to make cDNA of human D1HC protein having cysteine residue at carboxyl terminal express in E. coli, an expression vector was constructed. The DNA of plasmid pKHLD1HC having cDNA of human D1HC protein obtained in the above-mentioned x, was used as a template to carry out PCR. Used 5' primer is the primer used in the above-mentioned x and shown in SEQ ID NO: 17. Used 3' primer is shown in SEQ ID NO: 26. The primer is comprised of HindIII recognition sequence, sequence of termination codon, sequence coding for cysteine residue and the DNA sequence designed according to the sequence of the 330th-310th amino acids of SEQ ID NO: 23. PCR was carried out by using the primers of SEQ ID NO: 17 and SEQ ID NO: 26 to amplify DNA fragments. Thus amplified DNA fragments were cleaved with restriction enzymes KpnI and HindIII. The cleaved fragments was connected to KpnI-HindIII site of vector pKK233Kpn to give the expression vector pKHLD1HCcys. SEQ ID No: 27 shows the nucleotide sequence from initiation codon to termination codon coding for human D1HCcys protein contained in pKHLD1HCcys.

ζ . Production of human D1HCcys protein by using E. coli as host

E. coli JM 109 (made by TAKARA SHUZO Co.) was precultured which has the expression vector pKHLD1HCcys of human D1HCcys protein. Then, the precultured E. coli was inoculated in 100 mL of LB medium containing 10 mg of ampicilline, and shaking culture was carried out at 37°C in 500 mL of Sakaguchi flask until A_{600} of the medium containing E. coli became 0.3. Successively thereto was added IPTG so as to give a final concentration of 1 mM and further culture was continued for 6 hours. The cultured cells were collected by centrifugation, and suspended in 10 mL of 50 mM Tris-HCl buffer (pH 8.0) containing 50 mM EDTA, 5 % Triton X-100, 2 mM dithiothreitol and 8 % sucrose. Thereto was added lysozyme so as to give a final concentration of 0.1 %. The cell suspension was sonicated, and centrifuged to give insoluble inclusion bodies. The obtained inclusion bodies were dissolved with SDS and subjected to SDS-polyacrylamide gel electrophoresis to detect a band of molecular weight about 13,000.

η. Solubilization and renaturation of inclusion bodies containing human D1HCcys protein

All the inclusion bodies obtained in the above-mentioned ζ were dissolved in 10 ml of 50 mM Tris-HCl buffer (pH 7.4) containing 6M guanidine hydrochloride, 2 mM EDTA and 5 mM 2-mercaptoethanol, and centrifuged to give a supernatant. The supernatant was dialyzed against PBS to give a solution containing human D1HCcys protein. Thus prepared human D1HCcys protein in an amount ranging from 2.5-0 µg was mixed with 1 x 10⁵ Jurkat cells washed with the PBS containing 5 % BSA and 1 % glucose, and contained in 50 µl of the same buffer. Thereafter there was observed effect on rosette formation by adding 1 x 10⁷ sheep erythrocytes. Human D1HCcys protein dose-dependently inhibited the rosette formation of Jurkat cells with sheep erythrocytes.

Example 7

θ. Cloning of cDNA coding for human LFA-3

There was carried out cloning of cDNA coding for a full-length protein of human LFA-3. In the same manner as in the above-mentioned t, poly(A)⁺RNA from human T-cell line MOLT-4 was purified. Further the purified poly(A)⁺RNA was used to synthesize double strand cDNA.

From the double strand cDNA was amplified cDNA coding for human LFA-3 protein by using 2 kinds of primers synthesized in the above-mentioned s being shown in SEQ ID No: 14 and SEQ ID NO: 15 by PCR method in vitro under the same condition as in the above-mentioned u. After completing the reaction, size of PCR products was measured by a polyacrylamide gel electrophoresis, and then it was found that DNA fragments of about 800 base pairs were amplified. The DNA fragments of about 800 base pairs were extracted from the gel and treated with restriction enzyme PstI. Then, the treated DNA was inserted into PstI site of pUC19 vector (made by TAKARA SHUZO Co.) and cloned by using E. coli JM 109 as host.

Then, in order to sequence the DNA amplified by PCR, DNA fragment of about 800 base pairs cleaved with PstI was inserted into PstI site of M13mp19 phage vector (made by TAKARA SHUZO Co.) and determined by a conventional method using dideoxynucleotide triphosphates. As a result, cDNA sequence of human LFA-3 protein shown in SEQ ID NO: 16 was found among DNA fragments of about 800 base pairs. The amino acid sequence of human LFA-3 protein encoded by such cDNA sequence completely coincided with that of human LFA-3 (SEQ ID NO: 8) previously reported by B. P. Wallner et al. (Journal of Experimental Medicine, vol. 166, p 923, 1987).

ζ. Preparation of an expression vector of soluble human D1 protein having a part of amino acid sequence of D2 region in E. coli

In order to make DNA coding for soluble human D1 protein having the sequence from N-terminus to the first cysteine residue of D2 region (hereinafter referred to as "human D1cys protein") express in E. coli, an expression vector was constructed. As a template was used cDNA of human LFA-3 protein obtained in the above-mentioned θ to carry out PCR. Used 5' primer is the primer used in the above-mentioned x and shown in SEQ ID NO: 17. Used 3' primer is shown in SEQ ID NO: 28. The primer is comprised of HindIII recognition sequence, sequence of termination codon, sequence coding for cysteine residue and the DNA sequence designed according to the sequence of the 102nd-96th amino acids of SEQ ID NO: 8. PCR was carried out by using the primers of SEQ ID NO: 17 and SEQ ID NO: 28 to amplify DNA fragments. Thus amplified DNA fragments were cleaved with restriction enzymes KpnI and HindIII.

The cleaved fragment was connected to KpnI-HindIII site of vector pKK233Kpn to give an expression vector pKHLD1cys. SEQ ID NO: 29 shows the nucleotide sequence from initiation codon to termination codon coding for human D1cys protein contained in pKHLD1cys.

κ. Production of human D1cys protein by using E. coli as host

E. coli JM 109 (made by TAKARA SHUZO Co.) was precultured which has the expression vector pKHLD1cys of human D1cys protein. Then, the precultured E. coli was inoculated in 100 ml of LB medium containing 10 mg of ampicilline, and shaking culture was carried out at 37°C in 500 ml of Sakaguchi flask until A₆₀₀ of the medium containing E. coli became 0.3. Successively thereto was added IPTG so as to give a final concentration of 1 mM and further culture was continued for 6 hours. The cultured cells were collected by centrifugation, and suspended in 10 ml of 50 mM Tris-HCl buffer (pH 8.0) containing 50 mM EDTA, 5 % Triton X-100 and 8 % sucrose. Thereto was added lysozyme so as to give a final concentration of 1 %. The cell suspension was sonicated, and centrifuged to give an insoluble precipitation fraction. The obtained precipitation was washed with 50 % glycerol and successively with ethanol to give inclusion bodies containing produced human D1cys protein. The inclusion bodies were dissolved with SDS and subjected to SDS-polyacrylamide gel electrophoresis to detect a band of molecular weight about 12,000.

λ. Solubilization and renaturation of inclusion bodies containing human D1cys protein

All the inclusion bodies obtained in the above-mentioned β were dissolved in 10 mL of 50 mM Tris-HCl buffer (pH 7.4) containing 6M guanidine hydrochloride, 2 mM EDTA, and 5 mM 2-mercaptoethanol, and centrifuged to give a supernatant. The supernatant was dialyzed against PBS to give a solution containing human D1cys protein.

In addition to the ingredients used in the Examples, other ingredients can be used in the Examples as set forth in the specification to obtain substantially the same results.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) TITLE OF THE INVENTION: CELL ADHESION PROTEIN, GENE CODING FOR THE SAME, PROCESS FOR PREPARING THE SAME AND CARRIER ONTO WHICH THE SAME IS IMMOBILIZED

(ii) NUMBER OF SEQUENCES: 38

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 131 amino acids
(B) TYPE: amino acid

(vi) ORIGINAL SOURCE

(A) ORGANISM: Ovis
(G) CELL TYPE: leukocyte

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

Val Ser Gln Asp Ile Tyr Gly Ala Met Asn Gly Asn Val Thr Phe Tyr

1 5 10 15

Val Ser Gin Ser Gin Pro Phe Thr Glu Ile Met Trp Lys Lys Gly Lys

20 25 . . . 30

Asp Lys Val Val Glu Thr Asp Glu Thr Ser Gly Leu Glu Ala Phe Gln

35 40 45

Ser Phe Lys Asn Arg Val His Leu Asp Ile Val Ser Gly Asn Leu Thr

50 55 60

Ile Thr Gly Leu Thr Lys Leu Asp Glu Asp Val Tyr Glu Ile Glu Ser

65 70 75 80

Pro Ser Val Lys Lys Ser Ser Gln Phe His Leu Arg Val Ile Asp Tyr

26 27 28

Ala Arg His Arg Tyr Val Leu Phe Ala Ile Leu Pro Ala Val Ile Cys
 100 105 110
 5 Gly Leu Leu Phe Leu Lys Cys Phe Leu Gly Arg Arg Ser Gln Arg Asn
 115 120 125
 10 Ser Gly Pro
 130

(2) INFORMATION FOR SEQ ID NO: 2:

15 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 393 base pairs
- (B) TYPE: nucleic acid
- 20 (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

25 (ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: no

(vi) ORIGINAL SOURCE

- (A) ORGANISM Ovis
- (G) CELL TYPE: leukocyte

30 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

GTT TCC CAA GAT ATT TAT GGA GCT ATG AAT GGG AAT GTA ACC TTT TAC	48
Val Ser Gln Asp Ile Tyr Gly Ala Met Asn Gly Asn Val Thr Phe Tyr	
35 1 5 10 15	
GTT TCA GAG TCT CAA CCG TTT ACA GAG ATT ATG TGG AAG AAG GGG AAG	96
Val Ser Glu Ser Gln Pro Phe Thr Glu Ile Met Trp Lys Lys Gly Lys	
40 20 25 30	
GAT AAA GTT GTA GAA TGG GAT CAA ACA TCT GGA CTC GAA GCT TTT CAG	144
Asp Lys Val Val Glu Trp Asp Gln Thr Ser Gly Leu Gln Ala Phe Gln	
45 35 40 45	

TCT TTT AAA AAT AGA GTT CAT TTA GAC ATT GTG TCA GGT AAC CTC ACC	192
Ser Phe Lys Asn Arg Val His Leu Asp Ile Val Ser Gly Asn Leu Thr	
5 50 55 60	
ATC ACC GGG TTA ACA AAA TTA GAT GAA GAT GTG TAT GAA ATT GAA TCC	240
Ile Thr Gly Leu Thr Lys Leu Asp Glu Asp Val Tyr Glu Ile Glu Ser	
10 65 70 75 80	
CCA AGT GTT AAA AAG AGC TCC CAG TTC CAC CTC AGA GTG ATT GAT TAT	288
Pro Ser Val Lys Lys Ser Ser Gln Phe His Leu Arg Val Ile Asp Tyr	
15 85 90 95	
GCA AGG CAT AGG TAT GTG CTT TTT GCC ATA CTG CCA GCA GTA ATA TGT	336
Ala Arg His Arg Tyr Val Leu Phe Ala Ile Leu Pro Ala Val Ile Cys	
20 100 105 110	
GGC TTG CTG TTT TTA AAA TGT TTT CTG GGA CGT CGT AGC CAA CGA AAC	384
Gly Leu Leu Phe Leu Lys Cys Phe Leu Gly Arg Arg Ser Gln Arg Asn	
25 115 120 125	
TCA GGG CCC	393
Ser Gly Pro	
30 130	

35 (2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:

40 (A) LENGTH: 29 amino acids
 (B) TYPE: amino acids
 (D) TOPOLOGY: unknown

45 (ii) MOLECULE TYPE: peptide
 (iii) HYPOTHETICAL: yes
 (v) FRAGMENT TYPE: N-terminal peptide
 (ix) FEATURE: "Xaa" represents one of natural amino acids. The 12th is preferably Ser. The first is Val or Phe.
 The third is Glu or Ser.

50 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

Val/Phe Ser Gln/Ser Asp Ile Tyr Gly Ala Met Asn Gly Xaa Val Thr Phe Tyr	
1 5 10 15	
55 Val Ser Glu Ser Gln Pro Phe Thr Glu Ile Met Xaa Lys	
20 25	

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:

- 5 (A) LENGTH: 29 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

- 10 (ii) MOLECULE TYPE: other nucleic acid
 (A) DESCRIPTION: synthetic DNA
 (iii) HYPOTHETICAL: no
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

15 **GTTGGATCCT TYWSNCARGA YATHTAYGG**

29

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:

- 20 (A) LENGTH: 27 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

- 25 (ii) MOLECULE TYPE: other nucleic acid
 (A) DESCRIPTION: synthetic DNA
 (iii) HYPOTHETICAL: no
 (iv) ANTI-SENSE: yes
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

35 **ACACTGCAGC ATDATYTCTNG TRAANGG**

27

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:

- 40 (A) LENGTH: 24 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

- 45 (ii) MOLECULE TYPE: other nucleic acid
 (A) DESCRIPTION: synthetic DNA
 (iii) HYPOTHETICAL: no
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

50 **AGCTATGAAC GGGAAATGTAA CCTT**

24

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:

- 55 (A) LENGTH: 24 base pairs

- (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

ACCTTTTACG TTTCAGAGTC TCAA

24

(2) INFORMATION FOR SEQ ID NO: 8:

15 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 222 amino acids
 (B) TYPE: amino acid

20 (iii) HYPOTHETICAL: no
(vi) ORIGINAL SOURCE

(A) ORGANISM: *Homo sapiens*
(G) CELL TYPE: Peripheral Blood Lymphocytes

25 (x) PUBLICATION INFORMATION:

- (A) AUTHORS: Wallner, Barbara P.
Frey, Alexis Z.

30 (B) TITLE: Primary Structure of Lymphocyte Function-Associated Antigen 3 (LEA-3)
(C) JOURNAL: J. Exp. Med.
(D) VOLUME: 166
(F) PAGE: 923-932
(G) DATE: Oct-1987

(vi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

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Phe Ser Gla Gla Ile Tyr Gly Val Val Tyr Gly Asn Val Thr Phe His
 1 5 10 15

Val Pro Ser Asn Val Pro Leu Lys Glu Val Leu Trp Lys Lys Gln Lys
 20 25 30

10 Asp Lys Val Ala Gla Leu Glu Asn Ser Glu Phe Arg Ala Phe Ser Ser
 35 40 45

15 Phe Lys Asn Arg Val Tyr Leu Asp Thr Val Ser Gly Ser Leu Thr Ile
 50 55 60

Tyr Asn Leu Thr Ser Ser Asp Glu Asp Glu Tyr Glu Met Gla Ser Pro
 20 65 70 75 80

25 Asn Ile Thr Asp Thr Met Lys Phe Phe Leu Tyr Val Leu [Glu Ser Leu
 85 90 95

Pro Ser Pro Thr Leu Thr Cys Ala Leu Thr Asn Gly Ser Ile Glu Val
 30 100 105 110

Gln Cys Met Ile Pro Glu His Tyr Asn Ser His Arg Gly Leu Ile Met
 35 115 120 125

Tyr Ser Trp Asp Cys Pro Met Glu Gln Cys Lys Arg Asn Ser Thr Ser
 40 130 135 140

Ile Tyr Phe Lys Met Glu Asn Asp Leu Pro Gln Lys Ile Gln Cys Thr
 145 150 155 160

Leu Ser Asn Pro Leu Phe Asn Thr Thr Ser Ser Ile Ile Leu Thr Thr
 45 165 170 175

Cys Ile Pro Ser Ser] Gly His Ser Arg His Arg Tyr Ala Leu Ile Pro
 50 180 185 190

Ile Pro Leu Ala Val Ile Thr Thr Cys Ile Val Leu Tyr Met Asn Gly
 195 200 205

55 Ile Leu Lys Cys Asp Arg Lys Pro Asp Arg Thr Asn Ser Asn
 210 215 220

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:

- 5 (A) LENGTH: 32 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

- 10 (ii) MOLECULE TYPE: other nucleic acid
 (A) DESCRIPTION: synthetic DNA
 (iii) HYPOTHETICAL: no
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

15 TGGGGATCCA TGGTAAGTC~~A~~ AGATATT~~T~~T GG

32

(2) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:

- 20 (A) LENGTH: 36 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

- 25 (ii) MOLECULE TYPE: other nucleic acid
 (A) DESCRIPTION: synthetic DNA
 (iii) HYPOTHETICAL: no
 (iv) ANTI-SENSE: yes
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

30 TTTGTGACCC TGCA~~G~~C~~T~~AGG GCCCTGAGTT TCGTTG

36

(2) INFORMATION FOR SEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:

- 40 (A) LENGTH: 51 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

- 45 (ii) MOLECULE TYPE: other nucleic acid
 (A) DESCRIPTION: synthetic DNA
 (iii) HYPOTHETICAL: no
 (iv) ANTI-SENSE: yes
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

50 CAACTGCAGC TACGACGTCC CAGAAAACCT ATGCCTTGCA TAATCAATCA C

51

(2) INFORMATION FOR SEQ ID NO: 12:

(i) SEQUENCE CHARACTERISTICS:

- 5
 (A) LENGTH: 402 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: both

- 10
 (ii) MOLECULE TYPE: cDNA to mRNA
 (iii) HYPOTHETICAL: no
 (iv) ORIGINAL SOURCE

- 15
 (A) ORGANISM: Homo sapiens
 (H) CELL LINE: T cell line

- (vii) IMMEDIATE SOURCE:

- (B) CLONE: MOLT-4

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

20 TTT TCC CAA CAA ATA TAT GGT GTT GTG TAT GGG AAT GTA ACT TTC CAT 43

Phe Ser Gln Gln Ile Tyr Gly Val Val Tyr Gly Asn Val Thr Phe His

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25 GTA CCA AGC AAT GTG CCT TTA AAA GAG GTC CTA TGG AAA AAA CAA AAG 96

Val Pro Ser Asn Val Pro Leu Lys Glu Val Leu Trp Lys Lys Gln Lys

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30 GAT AAA GTT GCA CAA CTG GAA AAT TCT GAA TTC AGA GCT TTC TCA TCT 144

Asp Lys Val Ala Glu Leu Glu Asn Ser Glu Phe Arg Ala Phe Ser Ser

35

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45

35 TTT AAA AAT AGG GTT TAT TTA GAC ACT GTG TCA GGT AGC CTC ACT ATC 192

Phe Lys Asn Arg Val Tyr Leu Asp Thr Val Ser Gly Ser Leu Thr Ile

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TAC AAC TTA ACA TCA TCA GAT GAA GAT GAG TAT GAA ATG GAA TCG CCA 240
 Tyr Asn Leu Thr Ser Ser Asp Glu Asp Glu Tyr Glu Met Glu Ser Pro
 5 65 70 75 80
 AAT ATT ACT GAT ACC ATG AAG TTC TTT CTT TAT GTG CTT GGT CAT TCA 288
 Asn Ile Thr Asp Thr Met Lys Phe Phe Leu Tyr Val Leu Gly His Ser
 10 85 90 95
 AGA CAC AGA TAT GCA CTT ATA CCC ATA CCA TTA GCA GTA ATT ACA ACA 336
 Arg His Arg Tyr Ala Leu Ile Pro Ile Pro Leu Ala Val Ile Thr Thr
 15 100 105 110
 TGT ATT GTG CTG TAT ATG AAT GGT ATT CTG AAA TGT GAC AGA AAA CCA 384
 Cys Ile Val Leu Tyr Met Asn Gly Ile Leu Lys Cys Asp Arg Lys Pro
 20 115 120 125
 GAC AGA ACC AAC TCC AAT 402
 Asp Arg Thr Asn Ser Asn
 25 130

(2) INFORMATION FOR SEQ ID NO: 13:

- 30 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 134 amino acids
 (B) TYPE: amino acid
 35 (ii) MOLECULE TYPE: protein
 (iii) HYPOTHETICAL: yes
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

40 Phe Ser Gln Gln Ile Tyr Gly Val Val Tyr Gly Asn Val Thr Phe His
 1 5 10 15
 Val Pro Ser Asn Val Pro Leu Lys Glu Val Leu Trp Lys Lys Gln Lys
 45 20 25 30
 Asp Lys Val Ala Glu Leu Glu Asn Ser Glu Phe Arg Ala Phe Ser Ser
 50 35 40 45

Phe Lys Asn Arg Val Tyr Leu Asp Thr Val Ser Gly Ser Leu Thr Ile
 5 50 55 60

Tyr Asn Leu Thr Ser Ser Asp Glu Asp Glu Tyr Glu Met Glu Ser Pro
 10 65 70 75 80

Asn Ile Thr Asp Thr Met Lys Phe Phe Leu Tyr Val Leu | Gly His Ser
 15 85 90 95

Arg His Arg Tyr Ala Leu Ile Pro Ile Pro Leu Ala Val Ile Thr Thr
 100 105 110

Cys Ile Val Leu Tyr Met Asn Gly Ile Leu Lys Cys Asp Arg Lys Pro
 20 115 120 125

Asp Arg Thr Asn Ser Asn
 25 130

(2) INFORMATION FOR SEQ ID NO: 14:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 42 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(A) DESCRIPTION: synthetic DNA

(iii) HYPOTHETICAL: no

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

CATCACCTGC AGCCATGGAT GGTTGCTGGG AGCGACGCGG GG

42

(2) INFORMATION FOR SEQ ID NO: 15:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 41 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(A) DESCRIPTION: synthetic DNA

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

TTCACACTGC AGAATTCTCA ATTGGAGTTG GTTCTGTCTG G

41

(2) INFORMATION FOR SEQ ID NO: 16:

5

(i) SEQUENCE CHARACTERISTICS:

10

- (A) LENGTH: 753 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: both

(ii) MOLECULE TYPE: cDNA to mRNA

- (iii) HYPOTHETICAL: no
- (vi) ORIGINAL SOURCE

15

- (A) ORGANISM: Homo sapiens

(x) PUBLICATION INFORMATION:

20

- (A) AUTHORS: Wallner, Barbara P. Frey, Alexis Z.
- (B) TITLE: Primary Structure of Lymphocyte Function-Associated Antigen 3 (LFA-3)
- (C) JOURNAL: J. Exp. Med.
- (D) VOLUME: 166
- (F) PAGE: 923-932
- (G) DATE: Oct-1987

25

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

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ATG	GTT	GCT	GGG	AGC	GAC	GCG	GGG	CGG	GCC	CTG	GGG	GTC	CTC	AGC	GTG	48
Met	Val	Ala	Gly	Ser	Asp	Ala	Gly	Arg	Ala	Leu	Gly	Val	Leu	Ser	Val	
5	-28	-25				-20				-15						
GTC	TGC	CTG	CTG	CAC	TGC	TTT	GGT	TTC	ATC	AGC	TGT	TTT	TCC	CAA	CAA	96
Val	Cys	Leu	Leu	His	Cys	Phe	Gly	Phe	Ile	Ser	Cys	Phe	Ser	Gln	Gln	
10	-10				-5					1						
ATA	TAT	GGT	GTT	GTG	TAT	GGG	AAT	GTA	ACT	TTC	CAT	GTA	CCA	AGC	AAT	144
Ile	Tyr	Gly	Val	Val	Tyr	Gly	Asn	Val	Thr	Phe	His	Val	Pro	Ser	Asn	
15	5				10					15			20			
GTG	CCT	TTA	AAA	GAG	GTC	CTA	TGG	AAA	AAA	CAA	AAG	GAT	AAA	GTT	GCA	192
Val	Pro	Leu	Lys	Glu	Val	Leu	Trp	Lys	Lys	Gln	Lys	Asp	Lys	Val	Ala	
20					25					30			35			
GAA	CTG	GAA	AAT	TCT	GAA	TTC	AGA	GCT	TTC	TCA	TCT	TTT	AAA	AAT	AGG	240
Glu	Leu	Glu	Asn	Ser	Glu	Phe	Arg	Ala	Phe	Ser	Ser	Phe	Lys	Asn	Arg	
25					40					45			50			
GTT	TAT	TTA	GAC	ACT	GTG	TCA	GGT	AGC	CTC	ACT	ATC	TAC	AAC	TTA	ACA	288
Val	Tyr	Leu	Asp	Thr	Val	Ser	Gly	Ser	Leu	Thr	Ile	Tyr	Asn	Leu	Thr	
30					55					60			65			
TCA	TCA	GAT	GAA	GAT	GAG	TAT	GAA	ATG	GAA	TGG	CCA	AAT	ATT	ACT	GAT	336
Ser	Ser	Asp	Glu	Asp	Glu	Tyr	Glu	Met	Glu	Ser	Pro	Asn	Ile	Thr	Asp	
35					70					75			80			
ACC	ATG	AAG	TTC	TTT	CTT	TAT	GTG	CTT	GAG	TCT	CTT	CCA	TCT	CCC	ACA	384
Thr	Met	Lys	Phe	Phe	Leu	Tyr	Val	Leu	Glu	Ser	Leu	Pro	Ser	Pro	Thr	
40					85					90			95			100
CTA	ACT	TGT	GCA	TTG	ACT	AAT	GGA	AGC	ATT	GAA	GTC	CAA	TGC	ATG	ATA	432
Leu	Thr	Cys	Ala	Leu	Thr	Asn	Gly	Ser	Ile	Glu	Val	Gln	Cys	Met	Ile	
45					105					110			115			
CCA	GAG	CAT	TAC	AAC	AGC	CAT	CGA	GGA	CTT	ATA	ATG	TAC	TCA	TGG	GAT	480
Pro	Glu	His	Tyr	Asn	Ser	His	Arg	Gly	Leu	Ile	Met	Tyr	Ser	Trp	Asp	
50					120					125			130			

TGT CCT ATG GAG CAA TGT AAA CGT AAC TCA ACC AGT ATA TAT TTT AAG	528
Cys Pro Met Glu Gln Cys Lys Arg Ans Ser Thr Ser Ile Tyr Phe Lys	
5 135 140 145	
ATG GAA AAT GAT CTT CCA CAA AAA ATA CAG TGT ACT CTT AGC AAT CCA	576
Met Glu Asn Asp Leu Pro Gln Lys Ile Gln Cys Thr Leu Ser Asn Pro	
10 150 155 160	
TTA TTT AAT ACA ACA TCA TCA ATC ATT TTG ACA ACC TGT ATC CCA AGC	624
Leu Phe Asn Thr Thr Ser Ser Ile Ile Leu Thr Thr Cys Ile Pro Ser	
15 165 170 175 180	
AGC GGT CAT TCA AGA CAC AGA TAT GCA CTT ATA CCC ATA CCA TTA GCA	672
Ser Gly His Ser Arg His Arg Tyr Ala Leu Ile Pro Ile Pro Leu Ala	
20 185 190 195	
GTA ATT ACA ACA TGT ATT GTG CTG TAT ATG AAT GGT ATT CTG AAA TGT	720
Val Ile Thr Thr Cys Ile Val Leu Tyr Met Asn Gly Ile Leu Lys Cys	
25 200 205 210	
GAC AGA AAA CCA GAC AGA ACC AAC TCC AAT TGA	753
Asp Arg Lys Pro Asp Arg Thr Asn Ser Asn	
30 215 220	

35 (2) INFORMATION FOR SEQ ID NO: 17:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 38 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

40 (ii) MOLECULE TYPE: other nucleic acid

- (A) DESCRIPTION: synthetic DNA

45 (iii) HYPOTHETICAL: no

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

50 TTTGGTACCG GATGTTTCC CAACAAATAT ATGGTGTT

38

(2) INFORMATION FOR SEQ ID NO: 18:

55 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 42 base pairs
- (B) TYPE: nucleic acid

- (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

- 5 (ii) MOLECULE TYPE: other nucleic acid
 (A) DESCRIPTION: synthetic DNA
 (iii) HYPOTHETICAL: no
 (iv) ANTI-SENSE: yes
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18:

10 TTTAAGCTTG CTAGCTCAAT TGGAGTTGGT TCTGTCTGGT TT .

42

(2) INFORMATION FOR SEQ ID NO: 19:

- 15 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 72 base pairs
 (B) TYPE: nucleic acid
 20 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
 (ii) MOLECULE TYPE: other nucleic acid
 (A) DESCRIPTION: synthetic DNA
 25 (iii) HYPOTHETICAL: no
 (iv) ANTI-SENSE: yes
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 19:

30 ATCAAGCTTT CAATTGGAGT TGGTTCTGTC TGGTTTTCTG TCTCTGTGTC TTGAATGACC

60

AAGCACATAA AG

72

35 (2) INFORMATION FOR SEQ ID NO: 20:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 399 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
 (ii) MOLECULE TYPE: cDNA to mRNA
 (iii) HYPOTHETICAL: no
 (vi) ORIGINAL SOURCE
 (A) ORGANISM: Ovis
 (G) CELL TYPE: leukocyte
 50 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 20:

(2) INFORMATION FOR SEQ ID NO: 21:

(i) SEQUENCE CHARACTERISTICS:

- 5 (A) LENGTH: 321 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

- 10 (iii) HYPOTHETICAL: no
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 21:

ATG	GTA	AGT	CAA	GAT	ATT	TAT	GGA	GCT	ATG	AAT	GGG	AAT	GTA	ACC	TTT	48
Met	Val	Ser	Glu	Asp	Ile	Tyr	Gly	Ala	Met	Asn	Gly	Asn	Val	Thr	Phe	
1	5								10						15	
TAC	GTT	TCA	GAG	TCT	CAA	CCG	TTT	ACA	GAG	ATT	ATG	TGG	AAG	AAG	GGG	96
Tyr	Val	Ser	Glu	Ser	Gln	Pro	Phe	Thr	Glu	Ile	Met	Trp	Lys	Lys	Gly	
20									25						30	
AAG	GAT	AAA	GTT	GTA	GAA	TGG	GAT	CAA	ACA	TCT	GGA	CTC	GAA	GCT	TTT	144
Lys	Asp	Lys	Val	Val	Glu	Trp	Asp	Gln	Thr	Ser	Gly	Leu	Glu	Ala	Phe	
25									35						45	
CAG	TCT	TTT	AAA	AAT	AGA	GTT	CAT	TTA	GAC	ATT	GTG	TCA	GGT	AAC	CTC	192
Gln	Ser	Phe	Lys	Asn	Arg	Val	His	Leu	Asp	Ile	Val	Ser	Gly	Asn	Leu	
30									50						60	
ACC	ATC	ACC	GGG	TTA	ACA	AAA	TTA	GAT	GAA	GAT	GTG	TAT	GAA	ATT	GAA	240
Thr	Ile	Thr	Gly	Leu	Thr	Lys	Leu	Asp	Glu	Asp	Val	Tyr	Glu	Ile	Glu	
35									65						75	
TCC	CCA	AGT	GTT	AAA	AAG	AGC	TCC	CAG	TTC	CAC	CTC	AGA	GTG	ATT	GAT	288
Ser	Pro	Ser	Val	Lys	Ser	Ser	Gln	Phe	His	Leu	Arg	Val	Ile	Asp		
40									85						95	
TAT	GCA	AGG	CAT	AGG	TTT	TCT	GGG	ACG	TCG	TAG						321
Tyr	Ala	Arg	His	Arg	Phe	Ser	Gly	Tyr	Ser							
45									100						105	

55 (2) INFORMATION FOR SEQ ID NO: 22:

(i) SEQUENCE CHARACTERISTICS:

- 5
 (A) LENGTH: 408 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: both
 (D) TOPOLOGY: linear

- 10
 (ii) MOLECULE TYPE: cDNA to mRNA
 (iii) HYPOTHETICAL: no
 (vi) ORIGINAL SOURCE

- 15
 (A) ORGANISM: Homo sapiens
 (G) CELL TYPE: T cell line

- (vii) IMMEDIATE SOURCE:
 (B) CLONE: MOLT-4
 15 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 22:

ATG TTT TCC CAA CAA ATA TAT GGT GTT GTG TAT GGG AAT GTA ACT TTC 48

Met Phe Ser Gln Gln Ile Tyr Gly Val Val Tyr Gly Asn Val Thr Phe

1 5 10 15

CAT GTA CCA AGC AAT GTG CCT TTA AAA GAG GTC CTA TGG AAA AAA CAA 96

25 His Val Pro Ser Asn Val Pro Leu Lys Glu Val Leu Trp Lys Lys Gln

20 25 30

AAG GAT AAA GTT GCA GAA CTG GAA AAT TCT GAA TTC AGA GCT TTC TCA 144

Lys Asp Lys Val Ala Glu Leu Glu Asn Ser Glu Phe Arg Ala Phe Ser

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TCT	TTT	AAA	AAT	AGG	GTT	TAT	TTA	GAC	ACT	GTG	TCA	GGT	AGC	CTC	ACT	192	
5	Ser	Phe	Lys	Asn	Arg	Val	Tyr	Leu	Asp	Thr	Val	Ser	Gly	Ser	Leu	Thr	
	50						55							60			
10	ATC	TAC	AAC	TTA	ACA	TCA	TCA	GAT	GAA	GAT	GAG	TAT	GAA	ATG	GAA	TCG	240
	Ile	Tyr	Asn	Leu	Thr	Ser	Ser	Asp	Glu	Asp	Glu	Tyr	Glu	Met	Glu	Ser	
	65						70					75			80		
15	CCA	AAT	ATT	ACT	GAT	ACC	ATG	AAG	TTC	TTT	CTT	TAT	GTG	CTT	GGT	CAT	288
	Pro	Asn	Ile	Thr	Asp	Thr	Met	Lys	Phe	Phe	Leu	Tyr	Val	Leu	Gly	His	
	85						90							95			
20	TCA	AGA	CAC	AGA	TAT	GCA	CTT	ATA	CCC	ATA	CCA	TTA	GCA	GTA	ATT	ACA	336
	Ser	Arg	His	Arg	Tyr	Ala	Leu	Ile	Pro	Ile	Pro	Leu	Ala	Val	Ile	Thr	
	109						105							110			
25	ACA	TGT	ATT	GTG	CTG	TAT	ATG	AAT	GGT	ATT	CTG	AAA	TGT	GAC	AGA	AAA	384
	Thr	Cys	Ile	Val	Leu	Tyr	Met	Asn	Gly	Ile	Leu	Lys	Cys	Asp	Arg	Lys	
	115						120							125			
30	CCA	GAC	AGA	ACC	AAC	TCC	AAT	TGA								408	
	Pro	Asp	Arg	Thr	Asn	Ser	Asn										
	130						135										
35																	

(2) INFORMATION FOR SEQ ID NO: 23:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 333 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: both
 (D) TOPOLOGY: linear

(iii) HYPOTHETICAL: no

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 23:

ATG TTT TCC CAA CAA ATA TAT GGT GTT GTG TAT GGG AAT GTA ACT TTC 48
 Met Phe Ser Gln Gln Ile Tyr Gly Val Val Tyr Gly Asn Val Thr Phe
 5 1 5 10 15
 CAT GTA CCA AGC AAT GTG CCT TTA AAA GAG GTC CTA TGG AAA AAA CAA 96
 His Val Pro Ser Asn Val Pro Leu Lys Glu Val Leu Trp Lys Lys Glu
 10 20 25 30
 AAG GAT AAA GTT GCA GAA CTG GAA AAT TCT GAA TTC AGA GCT TTC TCA 144
 15 Lys Asp Lys Val Ala Glu Leu Glu Asn Ser Glu Phe Arg Ala Phe Ser
 35 35 40 45
 TCT TTT AAA AAT AGG GTT TAT TTA GAC ACT GTG TCA GGT AGC CTC ACT 192
 20 Ser Phe Lys Asn Arg Val Tyr Leu Asp Thr Val Ser Gly Ser Leu Thr
 50 50 55 60
 ATC TAC AAC TTA ACA TCA TCA GAT GAA GAT GAG TAT GAA ATG GAA TCG 240
 25 Ile Tyr Asn Leu Thr Ser Ser Asp Glu Asp Glu Tyr Glu Met Glu Ser
 65 65 70 75 80
 CCA AAT ATT ACT GAT ACC ATG AAG TTC TTT CTT TAT GTG CTT GGT CAT 288
 30 Pro Asn Ile Thr Asp Thr Met Lys Phe Phe Leu Tyr Val Leu Gly His
 85 85 90 95
 TCA AGA CAC AGA GAC AGA AAA CCA GAC AGA ACC AAC TCC AAT TGA 333
 35 Ser Arg His Arg Asp Arg Lys Pro Asp Arg Thr Asn Ser Asn
 100 100 105 110

40 (2) INFORMATION FOR SEQ ID NO: 24:

(i) SEQUENCE CHARACTERISTICS:

- 45 (A) LENGTH: 46 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

50 (ii) MOLECULE TYPE: other nucleic acid

- (A) DESCRIPTION: synthetic DNA

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 24:

55 TTTTTTCGAA CTGCAGCTAA CACGACGTCC CAGAAAAACCT ATGCCT

46

(2) INFORMATION FOR SEQ ID NO: 25:

(i) SEQUENCE CHARACTERISTICS:

- 5 (A) LENGTH: 324 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: both
 (D) TOPOLOGY: linear

- 10 (iii) HYPOTHETICAL: no
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 25:

ATG	GTA	AGT	CAA	GAT	ATT	TAT	GGA	GCT	ATG	AAT	GGG	AAT	GTA	ACC	TTT		48	
15	Met	[Val	Ser	Gln	Asp	Ile	Tyr	Gly	Ala	Met	Asn	Gly	Asn	Val	Thr	Phe		
	1				5				10				15					
TAC	GTT	TCA	GAG	TCT	CAA	CCG	TTT	ACA	GAG	ATT	ATG	TGG	AAG	AAG	GGG		96	
20	Tyr	Val	Ser	Glu	Ser	Gln	Pro	Phe	Thr	Glu	Ile	Met	Trp	Lys	Lys	Gly		
									20				25			30		
AAG	GAT	AAA	GTT	GTA	GAA	TGG	GAT	CAA	ACA	TCT	GGA	CTC	GAA	GCT	TTT		144	
25	Lys	Asp	Val	Val	Glu	Trp	Asp	Gln	Thr	Ser	Gly	Leu	Glu	Ala	Phe			
									35				40			45		
CAG	TCT	TTT	AAA	AAT	AGA	GTT	CAT	TTA	GAC	ATT	GTG	TCA	GGT	AAC	CTC		192	
30	Gln	Ser	Phe	Lys	Asn	Arg	Val	His	Leu	Asp	Ile	Val	Ser	Gly	Asn	Leu		
									50				55			60		
35	ACC	ATC	ACC	GGG	TTA	ACA	AAA	TTA	GAT	GAA	GAT	GTG	TAT	GAA	ATT	GAA		240
	Thr	Ile	Thr	Gly	Leu	Tyr	Lys	Leu	Asp	Glu	Asp	Val	Tyr	Glu	Ile	Glu		
									65				70			75		
TCC	CCA	AGT	GTT	AAA	AAG	AGC	TCC	CAG	TTC	CAC	CTC	AGA	GTG	ATT	GAT		288	
40	Ser	Pro	Ser	Val	Lys	Ser	Ser	Gln	Phe	His	Leu	Arg	Val	Ile	Asp			
									85				90			95		
TAT	GCA	AGG	CAT	AGG	TTT	TCT	GGG	ACG	TCG	TGT	TAG						324	
45	Tyr	Ala	Arg	His	Arg	(Phe)	Ser	Gly	Thr	Ser	Cys							
									100				105					

(2) INFORMATION FOR SEQ ID NO: 26:

(i) SEQUENCE CHARACTERISTICS:

- 5
(A) LENGTH: 37 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

- 10
(ii) MOLECULE TYPE: other nucleic acid
 (A) DESCRIPTION: synthetic DNA
(iii) HYPOTHETICAL: no
(iv) ANTI-SENSE: yes
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 26:

GGGGAAGCTT TCAACAAATTG GAGTTGGTTC TGTCTGG

37

- 15
(2) INFORMATION FOR SEQ ID NO: 27:

(i) SEQUENCE CHARACTERISTICS:

- 20
(A) LENGTH: 336 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: linear

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(iii) HYPOTHETICAL: no
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 27:

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	ATG TTT TCC CAA CAA ATA TAT GGT GTT GTG TAT GGG AAT GTA ACT TTC	48
5	Met [Phe Ser Gln Gln Ile Tyr Gly Val Val Tyr Gly Asn Val Thr Phe	
	1 5 10 15	
10	CAT GTA CCA AGC AAT GTG CCT TTA AAA GAG GTC CTA TGG AAA AAA CAA	96
	His Val Pro Ser Asn Val Pro Leu Lys Glu Val Leu Trp Lys Lys Gln	
	20 25 30	
15	AAG GAT AAA GTT GCA GAA CTG GAA AAT TCT GAA TTC AGA GCT TTC TCA	144
	Lys Asp Lys Val Ala Glu Leu Glu Asn Ser Glu Phe Arg Ala Phe Ser	
	35 40 45	
20	TCT TTT AAA AAT AGG GTT TAT TTA GAC ACT GTG TCA GGT AGC CTC ACT	192
	Ser Phe Lys Asn Arg Val Tyr Leu Asp Thr Val Ser Gly Ser Leu Thr	
	50 55 60	
25	ATC TAC AAC TTA ACA TCA TCA GAT GAA GAT GAG TAT GAA ATG GAA TCG	240
	Ile Tyr Asn Leu Thr Ser Ser Asp Glu Asp Glu Tyr Glu Met Glu Ser	
	65 70 75 80	
30	CCA AAT ATT ACT GAT ACC ATG AAG TTC TTT CTT TAT GTG CTT GGT CAT	288
	Pro Asn Ile Thr Asp Thr Met Lys Phe Phe Leu Tyr Val Leu Gly His	
	85 90 95	
35	TCA AGA CAC AGA GAC AGA AAA CCA GAC AGA ACC AAC TCC AAT TGT TGA	336
	Ser Arg His Arg Asp Arg Lys Pro Asp Arg Thr Asn Ser Asn Cys	
	100 105 110	
40		

(2) INFORMATION FOR SEQ ID NO: 28:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 35 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

- (A) DESCRIPTION: synthetic DNA

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 28:

TTAAGCTTC AACAAAGTTAG TGTGGGAGAT GGAAG

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5 (2) INFORMATION FOR SEQ ID NO: 29:

(i) SEQUENCE CHARACTERISTICS:

- 10 (A) LENGTH: 315 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: both
 (D) TOPOLOGY: linear

(iii) HYPOTHETICAL: no

15 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 29:

ATG	TTT	TCC	CAA	CAA	ATA	TAT	GGT	GTT	GTG	TAT	GGG	AAT	GTA	ACT	TTC	48
Met	Phe	Ser	Gln	Gln	Ile	Tyr	Gly	Val	Val	Tyr	Gly	Asn	Val	Thr	Phe	
1																
10																
CAT	GTA	CCA	AGC	AAT	GTG	CCT	TTA	AAA	GAG	GTC	CTA	TGG	AAA	AAA	CAA	96
His	Val	Pro	Ser	Asn	Val	Pro	Leu	Lys	Glu	Val	Leu	Trp	Lys	Lys	Gln	
20																
25																
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AAG GAT AAA GTT GCA GAA CTG GAA AAT TCT GAA TTC AGA GCT TTC TCA	144		
5 Lys Asp Lys Val Ala Glu Leu Glu Asn Ser Glu Phe Arg Ala Phe Ser			
35	40	45	
TCT TTT AAA AAT AGG GTT TAT TTA GAC ACT GTG TCA GGT AGC CTC ACT	192		
10 Ser Phe Lys Asn Arg Val Tyr Leu Asp Thr Val Ser Gly Ser Leu Thr			
50	55	60	
ATC TAC AAC TTA ACA TCA TCA GAT GAA GAT GAG TAT GAA ATG GAA TCG	240		
15 Ile Tyr Asn Leu Thr Ser Ser Asp Glu Asp Glu Tyr Glu Met Glu Ser			
65	70	75	80
CCA AAT ATT ACT GAT ACC ATG AAG TTC TTT CTT TAT GTG CTT GAG TCT	288		
20 Pro Asn Ile Thr Asp Thr Met Lys Phe Phe Leu Tyr Val Leu Glu Ser			
85	90	95	
CTT CCA TCT CCC ACA CTA ACT TGT TGA	315		
25 Leu Pro Ser Pro Thr Leu Thr Cys			
100			

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(2) INFORMATION FOR SEQ ID NO: 30:

(i) SEQUENCE CHARACTERISTICS:

- 35 (A) LENGTH: 675 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single

40 (ii) MOLECULE TYPE: cDNA to mRNA
 (iii) HYPOTHETICAL: no
 (vi) ORIGINAL SOURCE:

- (A) ORGANISM: Ovis
 (G) CELL TYPE: leukocyte

45 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 30:

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GTT TCC CAA GAT ATT TAT GGA GCT ATG AAT GGG AAT GTA ACC TTT TAC			48
Val Ser Gln Asp Ile Tyr Gly Ala Met Asn Gly Asn Val Thr Phe Tyr			
5	10	15	
GTT TCA GAG TCT CAA CCG TTT ACA GAG ATT ATG TGG AAG AAG GGG AAG			96
10	20	25	
Val Ser Glu Ser Gln Pro Phe Thr Glu Ile Met Trp Lys Lys Gly Lys			
20	25	30	
GAT AAA GTT GTA GAA TGG GAT CAA ACA TCT GGA CTC GAA GCT TTT CAG			144
15	Asp Lys Val Val Glu Trp Asp Gln Thr Ser Gly Leu Glu Ala Phe Gln		
35	40	45	
TCT TTT AAA AAT AGA GTT CAT TTA GAC ATT GTG TCA GGT AAC CTC ACC			192
20	Ser Phe Lys Asn Arg Val His Leu Asp Ile Val Ser Gly Asn Leu Thr		
50	55	60	
ATC ACC GGG TTA ACA AAA TTA GAT GAA GAT GTG TAT GAA ATT GAA TCC			240
25	Ile Thr Gly Leu Thr Lys Leu Asp Glu Asp Val Tyr Glu Ile Glu Ser		
65	70	75	80
CCA AGT GTT AAA AAG AGC TCC CAG TTC CAC CTC AGA GTG ATT GAA CCT			288
30	Pro Ser Val Lys Lys Ser Ser Gln Phe His Leu Arg Val Ile Glu Pro		
85	90	95	
CCT CCA ACA CCG TCA GCA TCT TGC TTC TTG ACT GAG GGT GGA AAC ATT			336
35	Pro Pro Thr Pro Ser Ala Ser Cys Phe Leu Thr Glu Gly Gly Asn Ile		
100	105	110	
ACT CTC ACC TGC TCG ATC CCG GAA GGT GAC CCC AAA GAG CTC GAT GAT			384
40	Thr Leu Thr Cys Ser Ile Pro Glu Gly Asp Pro Lys Glu Leu Asp Asp		
115	120	125	
AGT GAC CTA ATA CGG TAT TTG TGG GAA TGT CCG CCA ACA ATA CAG TGT			432
45	Ser Asp Leu Ile Arg Tyr Leu Trp Glu Cys Pro Pro Thr Ile Gln Cys		
130	135	140	

	CAC CGT GGC TCG ATT TCA TCT GAA GCC TTT GTC TCA GCG GAA AGT GAT	480		
	His Arg Gly Ser Ile Ser Ser Glu Ala Phe Val Ser Ala Glu Ser Asp			
5	145	150	155	160
	CTT TCA CAG AAT GTT CAG TGT ATC GTT AGC AAT CCA TTG TTC AGA ACA	528		
10	Leu Ser Gln Asn. Val Gln Cys Ile Val Ser Asn Pro Leu Phe Arg Thr			
	165	170	175	
	TCA GCT TCC GTC TCT TTG TCA ACC TGT TTG CCA GAG GAT TAT GCA AGG	576		
15	Ser Ala Ser Val Ser Leu Ser Thr Cys Leu Pro Glu Asp Tyr Ala Arg			
	180	185	190	
20	CAT AGG TAT GTG CTT TTT CCC ATA CTG CCA GCA GTA ATA TGT GGC TTG	624		
	His Arg Tyr Val Leu Phe Ala Ile Leu Pro Ala Val Ile Cys Gly Leu			
	195	200	205	
	CTG TTT TTA AAA TGT TTT CTG GGA CGT CGT AGC CAA CGA AAC TCA GGG	672		
25	Leu Phe Leu Lys Cys Phe Leu Gly Arg Arg Ser Gln Arg Asn Ser Gly			
	210	215	220	
	CCC			675
30	Pro			
	225			

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(2) INFORMATION FOR SEQ ID NO: 31:

(i) SEQUENCE CHARACTERISTICS:

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- (A) LENGTH: 225 amino acids
- (B) TYPE: amino acid

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(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 31:

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Val Ser Gln Asp Ile Tyr Gly Ala Met Asn Gly Asn Val Thr Phe Tyr
 1 5 10 15
 5 Val Ser Glu Ser Gln Pro Phe Thr Glu Ile Met Trp Lys Lys Gly Lys
 10 20 25 30
 10 Asp Lys Val Val Glu Trp Asp Gln Thr Ser Gly Leu Glu Ala Phe Gln
 15 35 40 45
 Ser Phe Lys Asn Arg Val His Leu Asp Ile Val Ser Gly Asn Leu Thr
 15 50 55 60
 Ile Thr Gly Leu Thr Lys Leu Asp Glu Asp Val Tyr Glu Ile Glu Ser
 20 65 70 75 80
 20 Pro Ser Val Lys Lys Ser Ser Gln Phe His Leu Arg Val Ile Glu Pro
 25 85 90 95
 Pro Pro Thr Pro Ser Ala Ser Cys Phe Leu Thr Glu Gly Gly Asn Ile
 25 100 105 110
 Thr Leu Thr Cys Ser Ile Pro Glu Gly Asp Pro Lys Glu Leu Asp Asp
 30 115 120 125
 Ser Asp Leu Ile Arg Tyr Leu Trp Glu Cys Pro Pro Thr Ile Gln Cys
 35 130 135 140
 His Arg Gly Ser Ile Ser Ser Gln Ala Phe Val Ser Ala Glu Ser Asp
 40 145 150 155 160
 Leu Ser Gln Asn Val Gln Cys Ile Val Ser Asn Pro Leu Phe Arg Thr
 45 165 170 175
 Ser Ala Ser Val Ser Leu Ser Thr Cys Leu Pro Glu Asp Tyr Ala Arg
 45 180 185 190

His Arg Tyr Val Leu Phe Ala Ile Leu Pro Ala Val Ile Cys Gly Leu		
195	200	205
Leu Phe Leu Lys Cys Phe Leu Gly Arg Arg Ser Gln Arg Asn Ser Gly		
210	215	220
Pio]		
225		

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(2) INFORMATION FOR SEQ ID NO: 32:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 33 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

- (A) DESCRIPTION: synthetic DNA

(iii) HYPOTHETICAL: no

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 32:

33

TTGGGGATCC ATGGTTTCCC AAGATATTAA TGG

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(2) INFORMATION FOR SEQ ID NO: 33:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 33 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

- (A) DESCRIPTION: synthetic DNA
(iii) HYPOTHETICAL: no
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 33:

50 ITGGGGATCC ATCGTAAGTC AAGATAATTTA TGG 33

(2) INFORMATION FOR SEQ ID NO: 34:

55

- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

- 5 (ii) MOLECULE TYPE: other nucleic acid
 (A) DESCRIPTION: synthetic DNA
 (iii) HYPOTHETICAL: no
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 34:

10 GTCGACCTGC AGCTACGACG TCCCAGAAAA CCTATG

36

- 15 (2) INFORMATION FOR SEQ ID NO: 35:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 597 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
- (ii) MOLECULE TYPE: cDNA to mRNA
- (iii) HYPOTHETICAL: no
- (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: Ovis
 - (G) CELL TYPE: leukocyte

- 20 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 35:

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GTT TCC CAA GAT ATT TAT GGA GCT ATG AAT GGG AAT GTA ACC TTT TAC	48
Val Ser Gln Asp Ile Tyr Gly Ala Met Asn Val Thr Phe Tyr	
5 1 5 10 15	
GTT TCA GAG TCT CAA CCG TTT ACA GAG ATT ATG TGG AAG AAG GGG AAG	96
Val Ser Gln Ser Gln Pro Phe Thr Glu Ile Met Trp Lys Lys Gly Lys	
10 20 25 30	
GAT AAA GTT GTA GAA TGG GAT CAA ACA TCT GGA CTC GAA GCT TTT CAG	144
Asp Lys Val Val Glu Trp Asp Gln Thr Ser Gly Leu Glu Ala Phe Gln	
15 35 40 45	
TCT TTT AAA AAT AGA GTT CAT TTA GAC ATT GTG TCA GGT AAC CTC ACC	192
Ser Phe Lys Asn Arg Val His Leu Asp Ile Val Ser Gly Asn Leu Thr	
20 50 55 60	
ATC ACC GGG TTA ACA AAA TTA GAT GAA GAT GTG TAT GAA ATT GAA TCC	240
Ile Thr Gly Leu Thr Lys Leu Asp Gln Asp Val Tyr Glu Ile Glu Ser	
25 65 70 75 80	
CCA AGT GTT AAA AAG AGC TCC CAG TTC CAC CTC AGA GTG ATT GAA CCT	288
Pro Ser Val Lys Lys Ser Ser Gln Phe His Leu Arg Val Ile Glu Pro	
30 85 90 95	
CCT CCA ACA CCG TCA GCA TCT TGC TTC TTG ACT GAG GGT GGA AAC ATT	336
Pro Pro Thr Pro Ser Ala Ser Cys Phe Leu Thr Glu Gly Gly Asn Ile	
35 100 105 110	
ACT CTC ACC TGC TCG ATC CCG GAA GGT GAC CCC AAA GAG CTC GAT GAT	384
Thr Leu Thr Cys Ser Ile Pro Glu Gly Asp Pro Lys Gln Leu Asp Asp	
40 115 120 125	

	AGT GAC CTA ATA CGG TAT TTG TGG GAA TGT CCG CCA ACA ATA CAG TGT	432
5	Ser Asp Leu Ile Arg Tyr Leu Trp Glu Cys Pro Pro Thr Ile Gln Cys	
	130 135 140	
	CAC CGT GGC TCG ATT TCA TCT GAA GCC TTT GTC TCA GCG GAA AGT GAT	480
10	His Arg Gly Ser Ile Ser Ser Glu Ala Phe Val Ser Ala Glu Ser Asp	
	145 150 155 160	
	CTT TCA CAG AAT GTT CAG TGT ATC GTT AGC AAT CCA TTG TTC AGA ACA	528
15	Leu Ser Glu Asn Val Gln Cys Ile Val Ser Asn Pro Leu Phe Arg Thr	
	165 170 175	
20	TCA GCT TCC GTC TCT TTG TCA ACC TGT TTG CCA GAG GAT TAT GCA AGG	576
	Ser Ala Ser Val Ser Leu Ser Thr Cys Leu Pro Glu Asp Tyr Ala Arg	
	180 185 190	
25	CAT AGG TTT TCT GGG ACG TCG	597
	His Arg Phe Ser Gly Thr Ser	
	195	

(2) INFORMATION FOR SEQ ID NO: 36:

(i) SEQUENCE CHARACTERISTICS:

35 (A) LENGTH: 199 amino acids
 (B) TYPE: amino acid

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: yes

40 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 36:

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Val Ser Gln Asp Ile Tyr Gly Ala Met Asn Gln Asn Val Thr Phe Tyr
 1 5 10 15
 Val Ser Glu Ser Gln Pro Phe Thr Glu Ile Met Trp Lys Lys Gly Lys
 20 25 30
 Asp Lys Val Val Glu Trp Asp Gln Thr Ser Gly Leu Glu Ala Phe Gln
 35 40 45
 Ser Phe Lys Asn Arg Val His Leu Asp Ile Val Ser Gly Asn Leu Thr
 50 55 60
 Ile Thr Glu Leu Thr Lys Leu Asp Gln Asp Val Tyr Glu Ile Glu Ser
 65 70 75 80
 Pro Ser Val Lys Lys Ser Ser Gln Phe His Leu Arg Val Ile Glu Pro
 85 90 95
 Pro Pro Thr Pro Ser Ala Ser Cys Phe Leu Thr Glu Gly Gly Asn Ile
 100 105 110
 Tyr Leu Thr Cys Ser Ile Pro Glu Gly Asp Pro Lys Glu Leu Asp Asp
 115 120 125
 Ser Asp Leu Ile Arg Tyr Leu Trp Glu Cys Pro Pro Thr Ile Gln Cys
 130 135 140
 His Arg Gly Ser Ile Ser Ser Gln Ala Phe Val Ser Ala Glu Ser Asp
 145 150 155 160
 Leu Ser Gln Asn Val Gln Cys Ile Val Ser Asn Pro Leu Phe Arg Thr
 165 170 175
 Ser Ala Ser Val Ser Leu Ser Thr Cys Leu Pro Glu Asp Tyr Ala Arg
 180 185 190
 His Arg Phe Ser Gly Thr Ser
 195

(2) INFORMATION FOR SEQ ID NO: 37:

50

(i) SEQUENCE CHARACTERISTICS:

55

- (A) LENGTH: 32 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(A) DESCRIPTION: synthetic DNA

(iii) HYPOTHETICAL: no

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 37:

5

TGGGGATCCA TGGTAAGTCA AGATATTAT GG

32

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(2) INFORMATION FOR SEQ ID NO: 38:

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(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 33 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

20

(ii) MOLECULE TYPE: other nucleic acid

(A) DESCRIPTION: synthetic DNA

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(iii) HYPOTHETICAL: no

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 38:

CCCCCTGCAGC TAGGGCCCTG AGTTTCGTTG GCT

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Claims

- 30 1. A sheep LFA-3 like protein having a structure of, from N-terminus, D1 region-TM region -C region and comprising the amino acid sequence of SEQ ID NO:1.
2. A human LFA-3 like protein having a structure of, from N-terminus, D1 region-TM region -C region and comprising the amino acid sequence of SEQ ID NO:13.
- 35 3. A gene coding for a sheep LFA-3 like protein according to claim 1, comprising the DNA sequence of SEQ ID NO:2.
4. A gene coding for a human LFA-3 like protein according to claim 2, comprising the DNA sequence of SEQ ID NO:12.
- 40 5. A soluble derivative of a sheep LFA-3 like protein according to claim 1 wherein amino acids 1 to 94 of SEQ ID NO: 1 are conserved and wherein at least one of amino acids 95 to 131 of SEQ ID NO:1 may be substituted or deleted.
6. A soluble derivative of a human LFA-3 like protein according to claim 2 wherein amino acids 1 to 94 of SEQ ID NO: 13 are conserved and wherein at least one of amino acids 95 to 134 of SEQ ID NO:13 may be substituted or deleted.
- 45 7. A soluble derivative of human LFA-3 like protein according to claim 2 wherein amino acids 1 to 93 of SEQ ID NO: 13 conserved and wherein at least one of amino acids 94 to 134 of SEQ ID NO:13 may be substituted or deleted.
8. A soluble derivative of a sheep LFA-3 like protein, the derivative having a structure of, from N-terminus, D-1 region -TM region -C region, containing at least one cysteine residue, and comprising the amino acid sequence of SEQ ID NO:25.
- 50 9. A soluble derivative of a human LFA-3 like protein, the derivative having a structure of, from N-terminus, D-1 region -TM region -C region, containing at least one cysteine residue, and comprising the amino acid sequence of SEQ ID NO:27.
10. A process for preparing a soluble derivative according to claim 5, 6 or 7 which comprises culturing a cell which is

transformed by a vector including a DNA coding for said protein and successively separating the produced protein.

11. A carrier onto which a soluble derivative according to claim 5, 6 or 7 is immobilized.
- 5 12. A process for preparing a soluble derivative according to claim 8 or 9, which comprises culturing a cell which is transformed by a vector including a DNA coding for said protein and successively separating the protein.

Patentansprüche

- 10 1. Ein Schaf-LFA-3-artiges Protein, welches eine Struktur vom N-Terminus an aus D1-Region-TM-Region-C-Region aufweist und die Aminosäuresequenz aus SEQ ID NO:1 umfaßt.
- 15 2. Human-LFA-3-artiges Protein, welches eine Struktur vom N-Terminus an, aus D1-Region-TM-Region-C-Region aufweist und die Aminosäuresequenz aus SEQ ID NO:13 umfaßt.
3. Gen, welches für ein Schaf-LFA-3-artiges Protein nach Anspruch 1 kodiert und die DNA-Sequenz aus SEQ ID NO:2 umfaßt.
- 20 4. Gen, welches für ein Human-LFA-3-artiges Protein nach Anspruch 2 kodiert und die DNA-Sequenz aus SEQ ID NO:12 umfaßt.
5. Lösliches Derivat eines Schaf-LFA-3-artigen Proteins nach Anspruch 1, wobei die Aminosäuren 1 bis 94 aus SEQ ID NO:1 erhalten sind und wobei mindestens eine der Aminosäuren 95 bis 131 aus SEQ ID NO:1 ersetzt oder 25 entfernt sein kann.
6. Lösliches Derivat eines Human-LFA-3-artigen Proteins nach Anspruch 2, wobei die Aminosäuren 1 bis 94 aus SEQ ID NO:13 erhalten sind und wobei mindestens eine der Aminosäuren 95 bis 134 aus SEQ ID NO:13 ersetzt oder entfernt sein kann.
- 30 7. Lösliches Derivat eines Human-LFA-3-artigen Proteins nach Anspruch 2, wobei die Aminosäuren 1 bis 93 aus SEQ ID NO:13 erhalten sind und wobei mindestens eine der Aminosäuren 94 bis 134 aus SEQ ID NO:13 ersetzt oder entfernt sein kann.
- 35 8. Lösliches Derivat eines Schaf-LFA-3-artigen Proteins, wobei das Derivat eine Struktur vom N-Terminus an aus D1-Region-TM-Region-C-Region aufweist, mindestens einen Cystein-Rest enthält und die Aminosäuresequenz aus SEQ ID NO:25 umfaßt.
- 40 9. Lösliches Derivat eines Human-LFA-3-artigen Proteins, wobei das Derivat eine Struktur vom N-Terminus an aus D1-Region-TM-Region-C-Region aufweist, mindestens einen Cystein-Rest enthält und die Aminosäuresequenz aus SEQ ID NO:27 umfaßt.
- 45 10. Verfahren zur Herstellung eines löslichen Derivats entsprechend Anspruch 5, 6 oder 7, welches Kultivieren einer Zelle, die mit einem Vektor transformiert wurde, der eine für dieses Protein kodierende DNA beinhaltet, und anschließendes Abtrennen des produzierten Proteins umfaßt.
11. Träger, auf dem ein lösliches Derivat nach Anspruch 5, 6 oder 7 immobilisiert ist.
- 50 12. Verfahren zur Herstellung eines löslichen Derivats nach Anspruch 8 oder 9, welches Kultivieren einer Zelle, die mit einem Vektor transformiert wurde, der eine für das Protein kodierende DNA beinhaltet, und anschließendes Abtrennen des Proteins umfaßt.

Revendications

- 55 1. Protéine de type LFA-3 de mouton, ayant la structure suivante, à partir de l'extrémité N-terminale, région D1 - région TM - région C, et comprenant la séquence d'aminoacides de SEQ ID n° 1.

2. Protéine de type LFA-3 humaine ayant la structure suivante, à partir de l'extrémité N-terminale, région D1 - région TM - région C, et comprenant la séquence d'aminoacides de SEQ ID n° 13.
3. Gène codant pour une protéine de type LFA-3 de mouton selon la revendication 1, comprenant la séquence d'ADN de SEQ ID n° 2.
4. Gène codant pour une protéine de type LFA-3 humaine selon la revendication 2, comprenant la séquence d'ADN de SEQ ID n° 12.
5. Dérivé soluble d'une protéine de type LFA-3 de mouton selon la revendication 1, dans lequel les aminoacides 1 à 94 de SEQ ID n° 1 sont conservés et dans lequel au moins l'un des aminoacides 95 à 131 de SEQ ID n° 1 peut être remplacé ou supprimé.
10. Dérivé soluble d'une protéine de type LFA-3 humaine selon la revendication 2, dans lequel les aminoacides 1 à 94 de SEQ ID n° 13 sont conservés et dans lequel au moins l'un des aminoacides 95 à 131 de SEQ ID n° 13 peut être remplacé ou supprimé.
15. Dérivé soluble de la protéine de type LFA-3 humaine selon la revendication 2, dans lequel les aminoacides 1 à 93 de SEQ ID n° 13 sont conservés et dans lequel au moins l'un des aminoacides 94 à 134 de SEQ ID n° 13 peut être remplacé ou supprimé.
20. Dérivé soluble d'une protéine de type LFA-3 de mouton, le dérivé ayant la structure suivante, à partir de l'extrémité N-terminale, région D-1 - région TM - région C, contenant au moins un résidu cystéine et comprenant la séquence d'aminoacides de SEQ ID n° 25.
25. Dérivé soluble d'une protéine de type LFA-3 humaine, le dérivé ayant la structure suivante, à partir de l'extrémité N-terminale, région D-1 - région TM - région C, contenant au moins un résidu cystéine et comprenant la séquence d'aminoacides de SEQ ID n° 27.
30. Procédé pour la production d'un dérivé soluble selon la revendication 5, 6 ou 7, comprenant la culture d'une cellule qui est transformée par un vecteur comprenant un ADN codant pour ladite protéine et ensuite la séparation de la protéine produite.
35. Support sur lequel est immobilisé un dérivé soluble selon la revendication 5, 6 ou 7.
40. Procédé pour la production d'un dérivé soluble selon la revendication 8 ou 9, comprenant la culture d'une cellule qui est transformée par un vecteur comprenant un ADN codant pour ladite protéine et ensuite la séparation de la protéine.

F/G. I

	10	20
human Phe Ser Gln Gln Ile Tyr Gly Val Val Tyr Gly Asn Val Thr Phe His Val Pro Ser Asn		
sheep Val Ser Gln Asp Ile Tyr Gly Ala Met Asn Gly Asn Val Thr Phe Tyr Val Ser Glu Ser		
	30	40
human Val Pro Leu Lys Glu Val Leu Trp Lys Lys Gln Lys Asp Lys Val Ala Glu Leu Glu Asn		
sheep Gln Pro Phe Thr Glu Ile Met Trp Lys Lys Gly Lys Asp Lys Val Val Glu Trp Asp Gln		
	50	
human -- Ser Glu Phe Arg Ala Phe Ser Ser Phe Lys Asn Arg Val Tyr Lys Asp Thr Val Ser		
sheep Thr Ser Gly Leu Glu Ala Phe Gln Ser Phe Lys Asn Arg Val His Leu Asp Ile Val Ser		
	60	70
human Gly Ser Leu Thr Ile Tyr Asn Leu Thr Ser Ser Asp Glu Asp Glu Tyr Glu Met Glu Ser		
sheep Gly Asn Leu Thr Ile Thr Gly Leu Thr Lys Leu Asp Glu Asp Val Tyr Glu Ile Glu Ser		
	80	90
human Pro Asn Ile Thr Asp Thr Met Lys Phe Phe Leu Tyr Val Leu Glu Ser Leu Pro Ser Pro		
sheep Pro Ser Val Lys Lys Ser Ser Gln Phe His Leu Arg Val Ile Glu Pro Pro Pro Thr Pro		
	100	110
human Thr Leu Thr Cys Ala Leu Thr Asn Gly----Ser Ile Glu Val Gln Cys Met Ile Pro Glu		
sheep Ser Ala Ser Cys Phe Leu Thr Glu Gly Gly Asn Ile Thr Leu Thr Cys Ser Ile Pro Glu		
	120	130
human His Tyr Asn Ser His Arg Gly-----Leu Ile Met Tyr Ser Trp Asp Cys Pro Met		
sheep Gly Asp Pro Lys Glu Leu Asp Asp Ser Asp Leu Ile Arg Tyr Leu Trp Glu Cys Pro Pro		
	140	150
human ---Glu Gln Cys Lys Arg-----Asn Ser Thr Ser Ile Tyr Phe---Lys Met Glu Asn		
sheep Thr Ile Gln Cys His Arg Gly Ser Ile Ser----Ser Glu Ala Phe Val Ser Ala Glu Ser		
	160	170
human Asp Leu Pro Gln Lys Ile Gln Cys Thr Leu Ser Asn Pro Leu Phe Asn Thr Thr Ser Ser		
sheep Asp Leu Ser Gln Asn Val Gln Cys Ile Val Ser Asn Pro Leu Phe Arg Thr Ser Ala Ser		
	180	190
human Ile Ile Leu Thr Thr Cys Ile Pro Ser Ser Gly His Ser Arg His Arg <u>Tyr Ala Leu Ile</u>		
sheep Val Ser Leu Ser Thr Cys Leu Pro----Glu Asp Tyr Ala Arg His Arg-----		
	200	210
human <u>Pro Ile Pro Leu Ala Val Ile Thr Thr Cys Ile Val Ile Tyr Met Asn Gly Ile Leu Lys</u>		
sheep -----		
	220	
human <u>Cys Asp Arg Lys Pro Asp Arg Thr Asn Ser Asn</u>		
sheep ---Phe Ser Gly Thr Ser		

FIG. 2

	10	20
human	Phe Ser Gln Gln Ile Tyr Gly Val Val Tyr Gly Asn Val Thr Phe His Val Pro Ser Asn	
	: : : : : : : : : : : : : : : :	
sheep	Val Ser Gln Asp Ile Tyr Gly Ala Met Asn Gly Asn Val Thr Phe Tyr Val Ser Glu Ser	
	30	40
human	Val Pro Leu Lys Glu Val Leu Trp Lys Gln Lys Asp Lys Val Ala Glu Leu Glu Asn	
	: : : : : : : : : : : : : : : :	
sheep	Gln Pro Phe Thr Glu Ile Met Trp Lys Gly Lys Asp Lys Val Val Glu Trp Asp Gln	
	50	
human	-- Ser Glu Phe Arg Ala Phe Ser Ser Phe Lys Asn Arg Val Tyr Ler Asp Thr Val Ser	
	: : : : : : : : : : : : : : : :	
sheep	Thr Ser Gly Leu Glu Ala Phe Gln Ser Phe Lys Asn Arg Val His Leu Asp Ile Val Ser	
	60	70
human	Gly Ser Leu Thr Ile Tyr Asn Leu Thr Ser Ser Asp Glu Asp Glu Tyr Glu Met Glu Ser	
	: : : : : : : : : : : : : : : :	
sheep	Gly Asn Leu Thr Ile Thr Gly Leu Thr Lys Leu Asp Glu Asp Val Tyr Glu Ile Glu Ser	
	80	90
human	<u>Pro Asn Ile Thr Asp Thr Met Lys Phe Phe Leu Tyr Val Leu Glu Ser Leu Pro Ser Pro</u>	
	: : : :	
sheep	Pro Ser Val Lys Lys Ser Ser Gln Phe His Leu Arg Val Ile -----	
	100	110
human	<u>Thr Leu Thr Cys Ala Leu Thr Asn Gly Ser Ile Glu Val Gln Cys Met Ile Pro Glu His</u>	
sheep	-----	
	120	130
human	<u>Tyr Asn Ser His Arg Gly Leu Ile Met Tyr Ser Trp Asp Cys Pro Met Glu Gln Cys Lys</u>	
sheep	-----	
	140	150
human	<u>Arg Asn Ser Thr Ser Ile Tyr Phe Lys Met Glu Asn Asp Leu Pro Gln Lys Ile Gln Cys</u>	
sheep	-----	
	160	170
human	<u>Thr Leu Ser Asn Pro Leu Phe Asn Thr Thr Ser Ser Ile Ile Leu Thr Thr Cys Ile Pro</u>	
sheep	-----	
	180	190
human	<u>Ser Ser Gly His Ser Arg His Arg Tyr Ala Leu Ile Pro Ile Pro Leu Ala Val Ile Thr</u>	
sheep	----- Asp Tyr Ala Arg His Arg Tyr Val Leu Phe Ala Ile Leu Pro Ala Val Ile ---	
	200	210
human	<u>Thr Cys Ile Val Leu Tyr Met Asn Gly Ile Leu Lys Cys ----- Asp Arg Lys Pro Asp</u>	
sheep	--- Cys Gly Leu Leu Phe ----- Leu Lys Cys Phe Leu Gly Arg Arg Ser Gln	
	220	
human	Arg Thr Asn Ser Asn	
sheep	Arg Asn Ser Gly Pro	

FIG. 3

